CANADIAN THESES ON MICROFICHE

THÈSES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Canadä

NL 339 (r. 85/09)

0-315-19514-2

National Library of Canada
Canadian Theses Division
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Division des thèses canadiennes

172

67422

PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

- Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur
David Richard Hampson

Date of Birth — Date de naissance
January 26, 1954

Country of Birth — Lieu de naissance
U.S.A.

Permanent Address — Résidence fixe
Pond Rd. Wolcott New York 14590

Title of Thesis — Titre de la thèse
The Monoamine Oxidase Inhibitor Tranylcypromine: A Comprehensive Neurochemical Study

University — Université
University of Alberta

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée
PhD

Year this degree conferred — Année d'obtention de ce grade
1984

Name of Supervisor — Nom du directeur de thèse
Dr. Glen B. Baker

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

Date
April 3, 1984

Signature
David R. Hampson

NL-91 (4/77)

THE UNIVERSITY OF ALBERTA

THE MONOAMINE OXIDASE INHIBITOR TRANYLCYPROMINE: A COMPREHENSIVE NEUROCHEMICAL STUDY

by

DAVID RICHARD HAMPSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES
(NEUROCHEMISTRY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES
AND FACULTY OF MEDICINE (PSYCHIATRY)

EDMONTON, ALBERTA

SPRING, 1984

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: David Richard Hampson

TITLE OF THESIS: The Monoamine Oxidase Inhibitor Tranylcypromine:
A Comprehensive Neurochemical Study

DEGREE FOR WHICH THESIS WAS PRESENTED: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1984

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(Signed) David R. Hampson

PERMANENT ADDRESS:

1984 Pond Road

Wolcott, New York 14590

U.S.A.

DATED April 11, 1984

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE MONOAMINE OXIDASE INHIBITOR TRANYLCYPROMINE: A COMPREHENSIVE NEUROCHEMICAL STUDY, submitted by David Richard Hampson in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

R T Coutts

Supervisor

Glen B. Baker

Supervisor

W. G. Dewhurst

Stephen R. Phelps

External Examiner

Date APRIL 11, 1984

DEDICATION

This thesis is dedicated to my parents, Richard and Jacqueline Hampson, and to my grandparents, Theodore and Alberta Ginther.

ABSTRACT

The antidepressant drug tranylcypromine is an inhibitor of the enzyme monoamine oxidase. This study has investigated a number of neurochemical properties that may be of importance to the antidepressant and other related effects of this drug.

The effects of long-term administration of a low dose of tranylcypromine on the levels of biogenic amines, including indolealkylamines, catecholamines, and trace amines, were studied in the rat. In the course of this work, novel analytical procedures for quantitation of tranylcypromine and 2-phenylethylamine in brain tissue were developed. Following daily administration of tranylcypromine, the brain levels of the trace amines and dopamine were elevated immediately and remained elevated at approximately the same level for up to 42 days. In contrast, 5-hydroxytryptamine and tranylcypromine itself increased steadily up to 21 days, after which only minimal increases were seen. At no time during the 42-day dosing period was there a trend towards a return to baseline levels. Both forms of monoamine oxidase were inhibited by approximately 87% after a single dose; after 42 days of tranylcypromine administration, enzyme inhibition reached 95%.

The commercially available preparation of tranylcypromine is a racemic mixture of (+)-tranylcypromine and (−)-tranylcypromine. The different neurochemical properties of these optical isomers were also investigated. The disposition of the tranylcypromine isomers in the brain was dramatically different; the (−)-isomer entered the brain faster, attained a higher level, and was cleared faster than the (+)-isomer. Both in vitro and in vivo monoamine oxidase inhibition assays were performed. The (+)-isomer was 20-30x more potent than the (-)-isomer in inhibiting monoamine oxidase, while (-)-tranylcypromine was a more potent inhibitor of amine reuptake and stimulator of amine release than was (+)-tranylcypromine. The results obtained in the present study agree very well with previous reports on the relative monoamine oxidase-inhibiting properties of the tranylcypromine isomers. The inhibiting properties on amine reuptake were also in general agreement with a previous report, although the differences between the isomers were less than those found by these other workers with the catecholamines. The results obtained in the present study also revealed that the tranylcypromine isomers, particularly the (-)-isomer, are also relatively strong reuptake inhibitors and releasers of 5-hydroxytryptamine.

The effects of acute administration of tranylcypromine isomers on the levels of phenylethylamine and 5-hydroxytryptamine (specific substrates for MAO-B and MAO-A respectively) in rat brain generally reflected the level of enzyme inhibition. The (+)-isomer significantly elevated both biogenic amines, while (-)-tranylcypromine significantly elevated only 5-hydroxytryptamine concentrations. The elevation of 5-hydroxytryptamine by (-)-tranylcypromine was apparent even with a level of monoamine oxidase inhibition as low as 40-50%.

The results of these studies reveal that the disposition and neurochemical properties of the (+)- and (-)-isomers of tranylcypromine are substantially different. It is likely that the effects of both isomers, although different, contribute to the overall antidepressant properties of tranylcypromine.

The metabolism of tranylcypromine has not been extensively studied. Using gas chromatography and combined gas chromatography-mass

spectrometry, a new metabolite (para-hydroxytranylcypromine) was detected in rat brain and urine. Tests for biological activity of this metabolite revealed that it possessed potency equal to or greater than tranylcypromine in inhibiting monoamine oxidase, inhibiting the reuptake of neurotransmitters, and in releasing neurotransmitters from striatal and hypothalamic tissue.

Finally, detection of the formation of 6-hydroxytetrahydro-β-carboline after the administration of tranylcypromine in combination with 5-hydroxytryptophan was achieved. 6-Hydroxytetrahydro-β-carboline is a member of a class of compounds whose pharmacological and neurochemical properties are diverse. The demonstration of the *in vivo* formation of this compound in the rat is a prerequisite for further studies which may attempt to determine the function of this class of compounds in the body. These results also indicate that the rat may be a suitable animal model for further studies.

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge my sincere appreciation to my two co-advisors, Dr. Glen B. Baker and Dr. Ronald T. Coutts. They have provided me with their expert advice and guidance, not only in the development and evolution of this thesis but in the development of my career as a neurochemist as well. I extend my appreciations to Dr. William G. Dewhurst and the Department of Psychiatry for their generosity in providing travel funds for me for the past three years. I am also grateful to the Faculty of Pharmacy and Pharmaceutical Sciences for providing me with a teaching assistantship.

Special thanks go to the following persons: Carolyn Farley for typing this manuscript, for her patience and advice on proper use of the English language; Dr. Donald LeGatt (University of Alberta Hospital), for the considerable time expended obtaining excellent mass spectral data; and to Dr. Tse Hall and Dr. Ronald Micetich (Faculty of Pharmacy and Pharmaceutical Sciences) for their useful advice in the area of pharmaceutical chemistry, and in particular for the substantial amount of time and effort spent on the successful development of procedures for the synthesis of para-hydroxytranylcypromine. I am also indebted to Dr. Robert Bornstein (Department of Psychiatry) for his invaluable advice on statistical procedures and to Deborah Kuefler for her assistance in the analysis of samples. I also wish to extend my thanks to Dr. Anthony Locock and Mr. Adil Nazarali for their consultation and their friendship.

Finally, and possibly most importantly, I would like to reiterate publically my deep appreciation to my fiancee, Tracy D. Horsman, for her never-ending support, loyalty, and love during this long and sometimes trying endeavour.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

A. Monoamine Oxidase: The Enzyme 2

B. Pharmacology of Monoamine Oxidase Inhibitors 6

C. Research Strategy: Tranylcypromine as a Model Monoamine Oxidase Inhibitor 13

1. Efficacy and Clinical Use 13

2. Acute Effects of Tranylcypromine on Neurotransmitter Systems 17

3. Long-Term Effects of Tranylcypromine on Neurotransmitter Systems 19

4. Differential Effects of Tranylcypromine Isomers 23

5. Metabolism of Tranylcypromine 27

6. Formation of Tetrahydro-β-Carbolines after the Administration of Tranylcypromine and 5-Hydroxytryptophan 30

7. Outline of Research Programme 38

(a) Acute and Chronic Administration of Tranylcypromine 38

(b) Administration of Tranylcypromine Isomers 40

(c) Detection and Characterization of Para-Hydroxytranylcypromine 41

(d) Detection of 6-Hydroxytetrahydro-β-Carboline 43

II. MATERIALS AND METHODS 45

A. Animals 46

B. Dosing Schedules 46

1. Acute and Chronic Dosing with Tranylcypromine 46

2. Tranylcypromine Isomer Studies 47

Chapter Page

3. Metabolism Studies 47

4. Tranylcypromine/5-Hydroxytryptophan Combination 48

C. Reagents 48

D. Instrumentation 51

1. Analysis of Biogenic Amines - General Considerations 51

2. Gas-Liquid Chromatography 54

3. Mass Spectrometry 55

4. Liquid Scintillation Spectrometry 55

5. Apparatus for Inhibition of Reuptake of Neurotransmitter Studies 55

6. Apparatus for the Study of Release of Neurotransmitters 56

7. Balances 56

8. Centrifugation 56

9. Miscellaneous Equipment 56

E. Drug Synthesis 58

1. 6-Hydroxytetrahydro-β-Carboline 58

2. _Para_-Hydroxytranylcypromine 58

F. Drug and Drug Metabolite Analyses 59

1. Tranylcypromine, Tranylcypromine Isomers, and Amphetamine 59

2. (a) _Para_-Hydroxytranylcypromine Analysis in Urine 60

(b) _Para_-Hydroxytranylcypromine Analysis in Brain 61

3. Analysis of 6-Hydroxytetrahydro-β-Carboline 62

G. Analysis of Biogenic Amines 62

1. Determination of Amines in Brain Tissue 62

(a) 5-Hydroxytryptamine and Tryptamine 62

Chapter Page

(b) Dopamine 64

(c) β-Phenylethylamine 64

2. Amine Determination in Urine 65

(a) 5-Hydroxytryptamine and Tryptamine 65

(b) Phenylethylamine and 3-Methoxytyramine 66

H. Monoamine Oxidase Assays 67

I. Determination of the Release of Tritiated Neurotransmitter Amines from Brain Tissue 68

J. Determination of the Inhibition of Reuptake of Tritiated Neurotransmitters from Brain Tissue 69

K. Statistical Analysis 70

III. RESULTS 72

A. Acute and Chronic Treatment with Tranylcypromine 72

1. Animal Weights 72

2. Levels of Tranylcypromine in the Brain 72

3. Inhibition of Monoamine Oxidase after Acute and Chronic Treatment with Tranylcypromine 74

4. Brain Levels of Biogenic Amines after Acute and Chronic Inhibition of Monoamine Oxidase 84

(a) β-Phenylethylamine and Tryptamine 84

(b) 5-Hydroxytryptamine 86

(c) Dopamine 86

5. Urinary Amine Levels after Acute and Chronic Treatment with Tranylcypromine 89

(a) β-Phenylethylamine and Tryptamine 89

(b) 5-Hydroxytryptamine and 3-Methoxytyramine 91

B. Administration of Tranylcypromine Isomers 93

1. Levels of Tranylcypromine Isomers in the Brain 93

Chapter Page

2. Inhibition of Monoamine Oxidase by Tranylcypromine Isomers 95

3. Levels of Biogenic Amines in Brain after Administration of Tranylcypromine Isomers 95

(a) β-Phenylethylamine 98

(b) 5-Hydroxytryptamine 98

4. The Effects of Tranylcypromine Isomers on the Inhibition of Reuptake of Tritiated Neurotransmitters 101

5. The Effects of Tranylcypromine Isomers on the Release of Tritiated Neurotransmitters 101

C. Detection and Characterization of Para-Hydroxytranylcypromine as a Metabolite of Tranylcypromine 104

1. Detection and Analysis of Para-Hydroxytranylcypromine 104

2. Biological Activities of Para-Hydroxytranylcypromine 113

(a) Inhibition of Monoamine Oxidase 113

(b) Inhibition of Reuptake of Tritiated Neurotransmitters by Para-Hydroxytranylcypromine 113

(c) Release of Tritiated Neurotransmitters by Para-Hydroxytranylcypromine 116

D. Detection of 6-Hydroxytetrahydro-β-Carboline 116

IV. DISCUSSION 122

A. Acute and Chronic Treatment with Tranylcypromine 122

1. Anorexiant Effects, Brain Tranylcypromine Concentrations, and Monoamine Oxidase Activity 122

2. Effects of Acute and Chronic Tranylcypromine Treatment on Levels of Biogenic Amines 128

B. Administration of Tranylcypromine Isomers 135

1. Disposition of and Monoamine Oxidase Inhibition by Tranylcypromine Isomers 135

Chapter Page

2. Response of Biogenic Amines to Tranylcypromine Isomers 139

3. Effects of Tranylcypromine Isomers on Inhibition of Reuptake and Release of Neurotransmitters 144

C. The Detection and Characterization of Para-Hydroxytranylcypromine 148

1. Detection of Para-Hydroxytranylcypromine 148

2. Characterization of the Biological Activity of Para-Hydroxytranylcypromine 153

D. Detection of 6-Hydroxytetrahydro-β-Carboline 155

V. CONCLUSIONS AND RECOMMENDATIONS 160

BIBLIOGRAPHY 164

APPENDIX I 180

APPENDIX II 181

LIST OF TABLES

Table	Description	Page
I	Classification of monoamine oxidase inhibitors	7
II	Platelet monoamine oxidase activity (Robinson *et al.*, 1978)	16
III	A list of derivatizing reagents used	49
IV	A list of chemicals and drugs used	50
V	Comparison of rat weights and weight gains in chronic saline- versus tranylcypromine-treated rats	73
VI	Monoamine oxidase activity after acute and chronic treatment with tranylcypromine	83
VII	Inhibition of monoamine oxidase *in vivo* by tranylcypromine isomers	96
VIII	Inhibition of monoamine oxidase *in vitro* by tranylcypromine isomers	97
IX	Inhibition of reuptake of tritiated neurotransmitters by tranylcypromine isomers	102
X	Release of tritiated neurotransmitters by tranylcypromine isomers	103
XI	Effects of pretreatment with iprindole on the levels of *para*-hydroxytranylcypromine in rat urine	109
XII	Effects of pretreatments with various psychotropic drugs on levels of tranylcypromine in rat brain	112
XIII	Comparison of *in vitro* monoamine oxidase inhibition by tranylcypromine and *para*-hydroxytranylcypromine	114
XIV	Inhibition of reuptake of tritiated neurotransmitters by *para*-hydroxytranylcypromine	115
XV	Release of tritiated neurotransmitters by *para*-hydroxytranylcypromine	117
XVI	A summary of the results of Willis and Smith (1982) demonstrating the anorexogenic effects of tranylcypromine	123

LIST OF FIGURES

Figure	Description	Page
1	Structures of some novel monoamine oxidase inhibitors under investigation for potential clinical use	9
2	Structures of tranylcypromine and the adduct of oxidized tranylcypromine	12
3	Hypothetical pathways for the metabolism of tranylcypromine	31
4	Condensation of indoleamines with aldehydes	32
5	Diagram of release apparatus	57
6	Electron impact mass spectrum of derivatized tranylcypromine	75
7	Electron impact mass spectrum of derivatized phenylethylamine	76
8	Electron impact mass spectrum of derivatized amphetamine	77
9	Chemical ionization mass spectrum of derivatized tranylcypromine	78
10	Chemical ionization mass spectrum of derivatized amphetamine	79
11	Chemical ionization mass spectrum of derivatized phenylethylamine	80
12	A chromatogram of a brain sample from a rat dosed with tranylcypromine	81
13	Levels of tranylcypromine in rat brain after acute and chronic treatment with tranylcypromine	82
14	Levels of phenylethylamine and tryptamine in rat brain after acute and chronic treatment with tranylcypromine	85
15	Levels of 5-hydroxytryptamine in rat brain after acute and chronic treatment with tranylcypromine	87
16	Levels of dopamine in rat brain after acute and chronic treatment with tranylcypromine	88

Figure	Description	Page
17	Urinary phenylethylamine and tryptamine after acute and chronic treatment with tranylcypromine	90
18	Urinary 5-hydroxytryptamine and 3-methoxytyramine after acute and chronic treatment with tranylcypromine	92
19	Levels of tranylcypromine isomers in rat brain after dosing with tranylcypromine isomers	94
20	Levels of phenylethylamine in rat brain after dosing with tranylcypromine isomers	99
21	Levels of 5-hydroxytryptamine in rat brain after dosing with tranylcypromine isomers	100
22	Electron impact mass spectrum of the pentafluorobenzoyl derivative of *para*-hydroxytranylcypromine	105
23	Chemical ionization mass spectrum of the pentafluorobenzoyl derivative of *para*-hydroxytranylcypromine in urine	106
24	A chemical ionization mass spectrum in the single ion monitoring mode of the pentafluorobenzoyl derivative of *para*-hydroxytranylcypromine in rat urine	107
25	An electron-impact mass spectrum of O-trifluoroacetyl, N-trifluoroacetyl, N-acetyl-*para*-hydroxytranylcypromine	110
26	An electron-impact mass spectrum in the single ion monitoring mode of O-trifluoroacetyl, N-trifluoroacetyl, N-acetyl-*para*-hydroxytranylcypromine in rat brain	111
27	An electron-impact mass spectrum of derivatized 6-hydroxy-tetrahydro-β-carboline	118
28	An electron-impact mass spectrum in the selected ion monitoring of a standard of derivatized 6-hydroxy-tetrahydro-β-carboline and urine samples from a control rat and a rat dosed with tranylcypromine and 5-hydroxytryptophan	119
29	The stereochemical relationship between the cis-trans isomers and the optical isomers of tranylcypromine	138

LIST OF ABBREVIATIONS

AA	Acetic anhydride
AMP	Amphetamine
AAD	Amino acid decarboxylase
BZD	Benzodiazepine
_p_-Cl-PEA	_Para_-chlorophenylethylamine
CI	Chemical ionization
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
DA	Dopamine
DEHPA	Di(2-ethylhexyl)phosphoric acid
E	Epinephrine
EDTA	Ethylenediaminetetraacetic acid
EI	Electron impact
FAD	Flavin adenine dinucleotide
GC	Gas chromatography
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
5-HTP	5-Hydroxytryptophan
HFBA	N-Heptafluorobutyric anhydride
HFBI	N-Heptafluorobutyrylimidazole
i.p.	Intraperitoneal
Ki	Dissociation constant
K_s	Half-maximal change
LSD	(+)-Lysergic acid diethylamide
MHIS	1-Methylhistamine
MS	Mass spectrometry
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MFO	Mixed-function oxidase
3-MT	3-Methoxytyramine
5-MT	5-Methyltryptamine
NE	Norepinephrine
nmr	Nuclear magnetic resonance
OA	Octopamine

_p_-OH-AMP	_Para_-hydroxyamphetamine
_p_-OH-TCP	_Para_-hydroxytranylcypromine
PEA	β-Phenylethylamine
PEOH	Phenylethanolamine
PLZ	Phenelzine
PFPA	Pentafluoropropionic anhydride
PFBZ	Pentafluorobenzoyl chloride
p.s.i.	Pounds per square inch
SAR	Structure-activity relationship
SIM	Single ion monitoring
T	Tryptamine
TA	Tyramine
TCA	Tricyclic antidepressant
TCP	(±)-Tranylcypromine
TCAA	Trichloracetic anhydride
TFAA	Trifluoroacetic anhydride
THBC	Tetrahydro-β-carboline

INTRODUCTION

I. INTRODUCTION

I.A. Monoamine Oxidase: The Enzyme

Monoamine oxidase (MAO, EC 1.4.3.4) is the primary catabolic enzyme involved in the inactivation of several biologically active aminergic neurotransmitters or neuromodulators. Substrates include dopamine (DA), norepinephrine (NE), epinephrine (E), 5-hydroxytryptamine (5-HT), and the "trace amines" (2-phenylethylamine [PEA], *m*- and *p*-tyramine [TA], phenylethanolamine [PEOH], *m*- and *p*-octopamine [OA], tryptamine [T], and methylhistamine [MHIS]). Monoamine oxidase is widely distributed in the animal kingdom, from lower invertebrates to mammals. In mammals, including humans, MAO is present in most tissues, with liver containing the highest concentration of any organ in the body. In the central nervous system (CNS), the highest concentrations are found in the hypothalamus, nucleus accumbens and the locus coeruleus, while cortex and cerebellum contain lower activity (Owen *et al.*, 1979). Activity varies by approximately a factor of three among these brain regions. Monoamine oxidase is found in both neurons and glial cells. It is thought to be primarily associated with the outer mitochondrial membrane; however, it is now accepted that a substantial amount is located extraneuronally (Cooper *et al.*, 1982). The relative importance of the extracellular portion of the enzyme is not known.

The general reaction catalysed by MAO is:

$$RCH_2NH_2 + O_2 + H_2O \longrightarrow RCHO + NH_3 + H_2O_2$$

Initial velocity and product inhibition studies have shown solubilized preparations of the enzyme to obey double-displacement (ping-pong) kinetics in which the enzyme reacts with amine substrate (in the unionized form) to form the aldehyde product and a reduced form of the enzyme. This enzyme form, in which the flavin component (flavin adenine dinucleotide, FAD) of the enzyme is reduced, then reacts with O_2 to regenerate the free enzyme and H_2O_2. Rat and human MAO require dietary riboflavin (as the source of the FAD cofactor) and Fe. The function of Fe in MAO is unknown, but liver and cardiac muscle MAO appear to be particularly susceptible to deficiencies in both Fe and riboflavin (Sourkes, 1979).

Using selective inhibitors, Johnston (1968) proposed that two forms of the enzyme exist. He designated them MAO-A and MAO-B. Experimental evidence supports the concept that these forms exist as two distinct enzymes embedded in a phospholipid bilayer, i.e. they are tightly bound, integral membrane proteins (Ekstedt and Oreland, 1975). The enzymatic activity of MAO-A is critically dependent on membrane phospholipids and this enzyme is therefore extremely difficult to solubilize; MAO-B activity is not dependent on membrane phospholipids and this enzyme is relatively easier to prepare in purified and solubilized form (White and Stine, 1982). The relative proportions of these two enzyme forms vary widely in different organs and species (Squires, 1972).

Differentiation of two forms of the enzyme is further substantiated by the identification of tissues which contain exclusively one form. For example, human blood platelets contain only MAO-B and certain cell lines grown in culture contain only MAO-A (Murphy et al., 1979). Similarly, studies of different populations of mitochondrial and synaptosomal MAO have suggested that MAO substrate and inhibitor properties reflect anatomical specialization (Student and Edwards, 1977). In addition, the differential sequence of appearance of the MAO forms during the first weeks of development in the rat have been interpreted as suggesting separate genetic regulation for the two enzyme forms.

Some substrates are not absolutely specific for either the A or B forms, and the relative specificity can be dependent upon substrate concentration. For example, PEA, usually considered a substrate for MAO-B, can be deaminated by MAO-A once the concentration of PEA has reached a critical level (Kinemuchi et al., 1979). At physiological concentrations, PEA appears to be deaminated solely by MAO-B. 5-HT displays more rigorous specificity, being oxidized by the A form even at relatively high concentrations. The classification of substrates as either MAO-A-specific or MAO-B-specific or mixed is undergoing revision. For example, NE has been classified as an MAO-A substrate, but recent evidence indicates that in humans it is a mixed substrate (Garrick and Murphy, 1982). Conversely, T has been classified as a mixed substrate, but in recent studies Bieck et al. (1984) have suggested a specificity towards MAO-A. Dopamine and TA are substrates for both forms of the enzyme (Suzuki et al., 1979).

The importance of MAO activity in human disease states is of course associated with its substrates. The amines deaminated by MAO have been postulated to be involved etiologically with a wide variety of conditions such as migraine, alcoholism, Lesch-Nyhan syndrome, and mental disorders. The most widely studied area in this regard is psychiatric diseases, particularly schizophrenia and the affective disorders. Although many parameters other than MAO activity may affect amine levels in vivo, variations in MAO activity may influence neurotransmitter levels and therefore may be involved in the etiology of some psychopathologies and in their alleviation by drugs.

The status of monoaminergic systems in humans has been assessed principally by (1) measurement of amine and amine metabolite levels in blood, urine and CSF, (2) analysis of postmortem tissue, and (3) measurement of platelet MAO activity. Platelets constitute a readily available tissue source for the direct measurement of MAO activity in humans. These cells also contain amine storage vesicles and transport (uptake) systems very similar to those found in neurons. The main drawback is that platelets contain only MAO-B. Platelet MAO activity differences range over 10-fold in normal individuals (Robinson and Nies, 1980), and a great many factors may influence this activity. Assay procedures, stress and anxiety, exercise, sex, and possibly age all affect MAO activity (Fowler et al., 1982a; Demisch et al., 1983). Also, several of the amines implicated in psychiatric disorders are primarily (DA, NE) or exclusively (5-HT) metabolized by MAO-A. The relationship between MAO-A and MAO-B and between platelet MAO activity and CNS MAO activity has not been thoroughly investigated. For these reasons Fowler et al. (1982a) have suggested that platelet MAO measurements may be of limited value for assessing brain monoaminergic systems, and that conclusions based on their measurement should be regarded with caution.

The major underlying premise of altered MAO activity as a factor in the etiology of psychiatric disorders is that changes in enzyme activity do indeed affect amine levels in the CNS. MAO is present in great excess, so that to induce elevations in amine levels, most of the enzyme must be inhibited before the therapeutic benefit of phenelzine treatment, for example, becomes apparent (Robinson et al., 1978; Raft et al., 1981).

To summarize, MAO is the major monoamine-degrading enzyme which is present in most tissues of all mammals and a wide variety of lower animals. It is present in two distinct forms, both of which are membrane bound, albeit to different degrees. The substrates for MAO have long been implicated in the etiology of affective disorders and therefore the endogenous activity of this enzyme and its activity after antidepressant therapies may be of paramount importance in understanding the endogenous biological parameters associated with these diseases and the mode of action of the drugs that alleviate the symptoms of the disease.

I.B. Pharmacology of Monoamine Oxidase Inhibitors

Various compounds, particularly arylalkylamines, are capable of inhibiting MAO. However, few of these compounds are actually potent enough to warrant pharmacological interest. The two major classifications of these drugs are the reversible and the irreversible MAO inhibitors (MAOIs), with the latter group being considerably more potent and in clinical use. A list of MAOIs and their classification is displayed in Table I.

Although the reversible MAOIs are not available on a commercial basis at the present time, interest in them is growing, and the use of this subclass of MAOIs, combined with the property of increased specificity (inhibition of MAO-A vs. MAO-B), may show considerable promise as effective and safe antidepressant drugs in the future (Ogren *et al.*, 1981; Bieck, P., personal communication). Ogren *et al.* (1981) state: "In our search for new and possibly clinically useful MAO inhibitors, we formulated the following criteria: (1) it should preferentially inhibit the A form of MAO, (2) it should be a reversible inhibitor to reduce the risk of overdosage and to avoid diminished selectivity following chronic treatment, (3) it should be a potent MAO inhibitor in the brain when

Table I. A list of various monoamine oxidase inhibitors used as antidepressants, as research drugs, or both.

	Nonspecific	MAO-A Specific	MAO-B Specific
IRREVERSIBLE	Tranylcypromine Phenelzine Nialamide Iproniazid Pheniprazine Isocarboxazid Isoniazid	Clorgyline	Pargyline[a] Deprenyl
REVERSIBLE	Amphetamine	Harmaline Harmine CGP-11305A[b] FLA 336[b] Cimoxatone[b]	MD 780236[b]

[a] Used primarily as antihypertensive

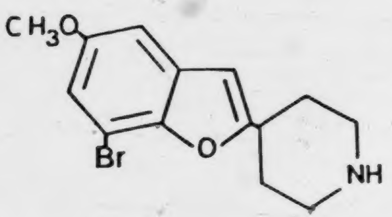
[b] See Figure 1 for chemical structures

given orally and exhibit a wide separation between MAO-inhibition and side effects, and (4) it should only weakly interact with tyramine." Several of the new reversible MAOIs that are being investigated for potential clinical use are shown in Figure 1.

The irreversible inhibitors are divided into three classes: the hydrazine type inhibitors, the propargylines, and the cyclopropylamines. The requirement of an aromatic moiety and a nitrogen atom suggests that in order to bind to the active site of the enzyme the inhibitor must bear some resemblance to the substrate. The best substrates for MAO contain a phenyl or indole moiety separated by one or two carbon atoms from the amine nitrogen. The initial enzyme-inhibitor interaction is relatively weak and reversible, but if other features are present the binding can become irreversible. These compounds are known as suicide inhibitors.

With the hydrazines, of which phenelzine (PLZ) is an example, the enzyme catalyzes the oxidation to produce phenylalkyldiazenes, which interact with the flavin cofactor of MAO to cause a potent irreversible inhibition (Patek and Hellerman, 1974). Kenney _et al._ (1979) concluded that in addition to the irreversible binding of arylalkyhydrazines to the flavin, part of the reactive phenylalkyldiazene reacts with a thiol residue near the active site, as is the case with the arylalkylamine TCP (see below). Although widely used, the hydrazines suffer from relatively poor penetration into the CNS and consequently a high incidence of peripheral side effects. The hydrazine MAOIs are not specific for MAO-A or MAO-B.

The propargylamines, on the other hand, show remarkable specificity. This group includes the MAO-A specific inhibitor clorgyline, and

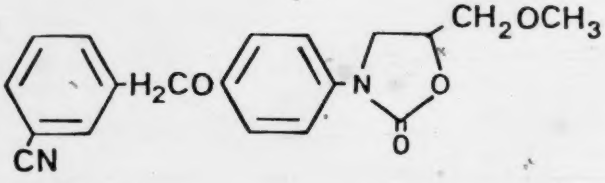


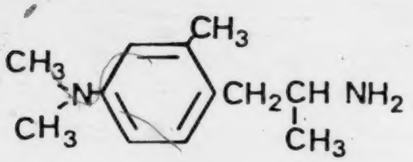
CGP 11305A

Reversible,MAO-A selective

CIMOXATONE

Revesible; MAO-A selective





FLA 336

Reversible; MAO-A selective

MD 780236

Reversible; MAO-B selective

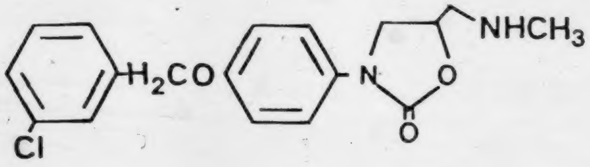


Figure 1. Structures of some novel monoamine oxidase inhibitors under investigation for potential clinical use.

the MAO-B specific inhibitors deprenyl and pargyline, the former being the more specific. The propargyline MAOIs are acetylenic compounds, which, after initial reversible binding to the substrate site, cause a reduction in the flavin cofactor of MAO, which in turn results in the drug becoming covalently bound via a flavocyanine adduct (Hellerman and Erwin, 1968). The specificity may be the result of both the differential molecular dimensions and the lipophilicity of the inhibitors (Maxwell and White, 1978). The distance between the nitrogen atom and the aromatic ring is greater in the extended conformation of clorgyline than in deprenyl or pargyline. The corresponding distances in the MAO-A substrates 5-HT and T are also longer than in the phenylethylamine substrates. In addition, the chlorine substitution on the clorgyline molecule enhances lipophilicity. This is consistent with the observation that MAO-A is critically dependent upon the associated phospholipids of the membrane complex, while the MAO-B form is not (White and Stine, 1982).

Fowler _et_ _al._ (1982b) have examined the kinetics of MAO inhibition in some detail. Their results have indicated that the major factors influencing selectivity for clorgyline and deprenyl are different. The dissociation constant (Ki) value for the reversible interaction between clorgyline and MAO-A is about 1000 times lower than that towards MAO-B, and this difference is sufficient to account for most, but not all, of the selectivity of this compound. The Ki value of MAO-B towards deprenyl is only 40-fold lower than that towards the A form of the enzyme. However, in this case, the rate of formation of the irreversible adduct is considerably faster for the B form than the A form and in the case of deprenyl, makes a major contribution to the selectivity of the inhibitor. Thus the selectivity of these compounds is not associated with different modes of irreversible binding to the FAD, but rather to different recognition sites near the active site. This would account for the kinetic differences observed in the formation of the adduct.

The third class of irreversible inhibitors are the arylcyclopropylamines, which include tranylcypromine (TCP), the subject of the present study. These are among the most potent MAOIs. As with the acetylenic (propargyl) inhibitors, the initial loss of enzyme activity is reversible and competitive with substrate; this is followed by gradual irreversible inactivation (Paech _et al._, 1979). Paech _et al._ (1980) have studied the reaction of TCP with MAO in considerable detail. They have concluded that TCP is oxidized by flavin to reactive nucleophilic species, which are either a ketone, an imine, or both. These reactive species then combine with a sulfhydryl group on a cysteine residue located on the substrate binding site to form a thioaminoketal or a thiohemiketal (Figure 2). These covalently bonded species are stabilized by noncovalent interactions between the enzyme and the phenyl group of TCP. The proposed structure of the suicide-inactivated enzyme is very similar to that advanced for the inactivation mechanism of cyclopropanone hydrate by the NAD-dependent enzyme aldehyde dehydrogenase (Singer _et al._, 1981).

The fact that the flavin is not the component alkylated was concluded primarily from two lines of evidence. First, on proteolysis of the alkylated (radiolabelled) enzyme at neutral pH and under anaerobiosis (conditions under which N-alkylated adducts are not likely to break) a flavin peptide containing no radioactivity was obtained. Second, upon gradual denaturation of the inactivated enzyme, reoxidation of the fla-

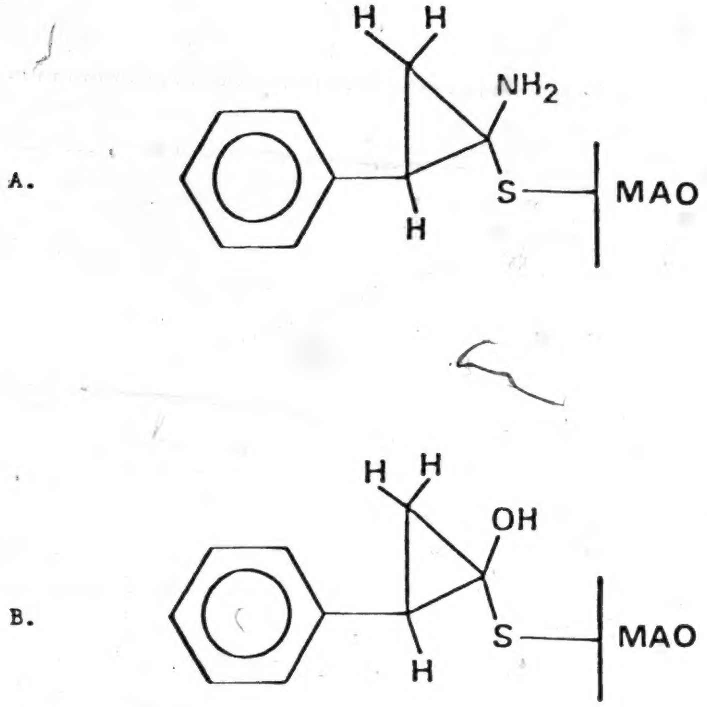

A.
H
H
NH_2
S
MAO
H
B.
H
H
OH
S
MAO
H

Figure 2. Possible structures of the adduct of oxidized tranylcypromine and a cysteine residue of monoamine oxidase. A. Thioaminoketal. B. Thiohemiketal. (From Paech _et al._, 1980).

vin was far faster than release of the radioactive inhibitor (Paech et al., 1979). Thus the mechanism outlined above was proposed as the most likely explanation.

I.C. Research Strategy: Tranylcypromine as a Model Monoamine Oxidase Inhibitor

1. Efficacy and Clinical Use

Tranylcypromine (Parnate®) and phenelzine (Nardil®) together constitute the two most widely used MAOIs at the present time. Between 1959 and 1964, TCP was a popular and widely used antidepressant drug. In 1964, the U.S. Food and Drug Administration ordered its removal from the market for a few months after reports, mostly in British journals, of severe hypertensive reactions in some patients. Its use in the U.S. was reinstated under strict guidelines. Although the use of TCP, and MAOIs in general, is not as extensive as that of other types of antidepressant drugs, interest in them is increasing again. This revival is the result of the realization that their deleterious side effects may have been overestimated and that they may be the most efficacious drugs in certain types of depression, particularly depressions associated with anxiety, agitation, and phobias (Lesse, 1978; Sheehan et al., 1980; Tyrer, 1976) and anergia (Himmelhoch et al., 1982).

The efficacy of TCP as an antidepressant has been extensively documented (see Quitkin et al., 1979 for review). Lesse (1978) reported on clinical trials with TCP in 1000 patients, most with severe agitated depressions. He concluded that TCP was a safe and highly effective antidepressant with a rapid onset of action. It may be the drug of choice, particularly in those cases which are dominated by suicidal ideation where a rapid therapeutic effect is imperative. Curiously, Lesse found that the antidepressant effect was substantially potentiated by combining TCP with a neuroleptic such as trifluperazine (Stelazine®). No explanation was given for the synergistic effect. In this regard, it is noteworthy that at least one neuroleptic, chlorpromazine, produces a down-regulation of α-adrenoceptor-stimulated cAMP, a property which has been hypothesized as a mode of action of antidepressants (Sugrue, 1983). In addition, Yasensky et al. (1982) found that phenothiazines blocked ring hydroxylation of PEA, an amine closely related to TCP structurally. It is conceivable that trifluoperazine could be acting on TCP in a similar manner, resulting in increased brain levels of TCP.

As with most other MAOIs, side effects do occur with TCP. Orthostatic hypotension is the major deleterious side effect most often encountered. Alopecia, weight gain, and hypertensive crisis are reported less frequently. The incidence of hypertensive crisis, although potentially serious, has been overestimated. The cause is attributed to the ingestion of tyramine-containing foods (e.g. certain cheeses, wines, beer) during treatment with MAOIs. Tyramine, which is normally metabolized via MAO in the intestine and liver, is elevated to levels which cause it to displace NE from storage vesicles, thus resulting in hypertension. Lesse et al. (1978) reported an incidence of 0.3% in their study of 1000 patients and Himmelhoch et al. (1982) reported no incidence of hypertensive crisis in their study of 59 patients.

Tranylcypromine and all other MAOIs are also potent suppressors of REM sleep. The physiological consequences of this have not been adequately assessed, although REM suppression is a phenomenon common to tricyclic antidepressants (TCAs) and has been proposed as the mechanism of action of antidepressant drugs (Vogel, 1983).

In order to be effective antidepressants, MAOIs must be given in sufficient dosage to produce a critical level of MAO inhibition. Robinson *et al.* (1978) studied the relationship between platelet MAO inhibition with phenelzine and clinical improvement. The results of this study are shown in Table II. These data indicate that the higher the level of MAO inhibition, the greater the likelihood of improvement. Minimum required levels of MAO inhibition are now widely accepted as being in the range of 80-90% (based on platelet MAO-B activity).

In a clinical trial of the MAO-B-specific inhibitor deprenyl, Mendlewicz and Youdim (1983) found that inhibition of platelet MAO by 90% was required for effective therapy. Patients with less than 90% inhibition did not respond.

Quitkin *et al.* (1979) suggested that the usual effective dose of TCP was 40 to 60 mg/day. They also suggested that several clinical trials comparing TCP with tricyclic antidepressants used inadequate dosages of MAOIs (Quitkin *et al.*, 1979). However, Simpson *et al.* (1983) found that a single 10 mg dose of TCP caused almost 80% inhibition of platelet enzyme in humans, indicating that substantially less drug than that suggested by Quitkin *et al.* (1979) may be effective. No studies have examined platelet MAO activity versus clinical improvement with TCP.

In summary, TCP has been shown to be a safe, fast-acting, and effective antidepressant. It appears to be most efficacious in patients with anergia, anxiety, and agitation. Tranylcypromine may be contraindicated in patients with inherently low blood pressure. The

Table II. Comparison of platelet monoamine oxidase activity with the percentage of depressed patients responding to phenelzine treatment (from Robinson *et al.*, 1978).

% Inhibition of Platelet MAO Activity	% Responders
< 80	44
80	68
90	79

incidence of hypertensive crisis resulting from TCP administration is apparently low and may be totally obviated by restricting a selected few foods from the diet.

2. Acute Effects of Tranylcypromine on Aminergic Neurotransmitter Systems

Tranylcypromine is a nonselective MAOI and therefore increases levels of monoamines regardless of their specificity for MAO-A or MAO-B. Elevated levels of amines are apparent throughout the body within an hour after administration, i.e. TCP acts centrally and peripherally. Levels of 5-HT are increased on a proportionately greater basis than those of the catecholamines, and the trace amines are increased to a much greater extent above their baseline values than are the catecholamines or 5-HT (McKim et al., 1980; Philips and Boulton, 1979). The dramatic increases in the trace amines are a consequence of their high turnover rates.

In addition to its MAO-inhibiting properties, TCP also inhibits the reuptake of catecholamines (Escobar et al., 1974) and causes release of NE (Schildkraut, 1970), DA and 5-HT (Baker et al., 1980). These effects, particularly the releasing properties, may contribute to the stimulating effects seen at higher doses. The property of inhibiting the reuptake of neurotransmitters is characteristic of most of the classic TCAs. All these properties combined probably contribute to the clinical efficacy of TCP.

After the initial increases in the amine levels occur, secondary short-term adaptations take place, at least in the case of the catecholamines. These include a reduction in amine synthesis via a feedback mechanism on the rate-limiting synthetic enzyme, tyrosine hydroxylase in some brain areas (see Section I.C.3). This enzyme controls the synthesis of both DA and NE. A feedback mechanism on tryptophan hydroxylase, the synthetic enzyme for 5-HT, has not been conclusively demonstrated.

As neuronal amines concentrations increase, other amines e.g. trace amines) accumulating in the cytoplasm begin to enter storage vesicles, where it has been postulated they remain to be released as cotransmitters or false transmitters or from which they may displace endogenous amines (Murphy, 1972). These changes in vesicular amines available for release are incompletely understood, but the altered proportions of different amines (e.g. trace amines vs. DA, NE, or 5-HT) may influence the postsynaptic response.

In addition to these changes in the balance of amines within neurons, other delayed responses occur within a period of hours. Slowing of neuronal firing rate has been demonstrated in both 5-HT-containing neurons in the median raphe (Aghajanian, 1972) and in NE-containing cells in the locus coeruleus (Campbell _et al._, 1979). Both effects are thought to represent a direct consequence of increased amine accumulations. With longer term administration of MAOIs, other postsynaptic changes occur (see Section I.C.3) which appear to be additional dampening responses to the increased neuronal activity initially produced by MAO inhibition.

As stated above, the trace amines respond dramatically to MAO inhibition. These amines have been coined neuromodulators (Boulton, 1976) since it has been demonstrated that they can potentiate the postsynaptic actions of iontophoretically applied NE and DA (Jones and Boulton, 1980a) and 5-HT (Jones and Boulton, 1980b). However, pharmacological evidence (Dewhurst, 1968) and in vitro binding studies (Hauger, 1982; Cascio and Keller, 1983) indicate that there may be specific receptors for PEA and tryptamine, and a transmitter role for these substances cannot be ruled out.

The possible association of the trace amines and affective disorders as first proposed by Dewhurst (1968) is supported not only by their dramatic response to TCP and other MAOIs, but also by reports of altered levels during depressed states (Sabelli et al., 1983) and stressful periods (Paulos and Tessel, 1982).

In addition, the trace amines may be valuable in determining the extent of MAO inhibition during treatment with MAOIs. Bieck and Antonin (1982) studied urinary excretion of T in humans after administration of TCP. They found T excretion increased 7-fold during a 25 mg/day regimen. Tryptamine values returned to control levels 4 days after cessation of TCP. The possibility of using urinary T excretion as a convenient method for analysis of drug efficacy and compliance was discussed. Tryptamine is of interest not only because of its own properties, but also because of the potential for its conversion to the psychotomimetic substance dimethyltryptamine via indoleamine-N-methyltransferase (Wyatt et al. 1973). Whether or not this reaction is of any significance under normal conditions or during MAOI administration is equivocal.

3. Long-Term Effects of Tranylcypromine on Neurotransmitter Systems

After acute administration of TCP, monoamine levels rise immediately. What happens after continuous daily administration as in a therapeutic situation? Pre- and post-synaptic changes may both occur. Particularly relevant are the dynamics of neurotransmitter systems after

10-20 days of administration, which is generally accepted as the time required for mood elevation to become apparent in depressed patients treated with a variety of antidepressant treatments.

Robinson et al. (1979) studied the effects of chronic administration of PLZ and TCP on rat brain monoamine levels. They reported peak increases in NE, DA, and 5-HT between 3 and 7 days after the beginning of treatment followed by a gradual decline to baseline levels after 14 days of treatment. Using 7.5 mg/kg or 15 mg/kg of phenelzine, or 5 mg/kg TCP, 5-HT levels were maximum at 7 days, with increases ranging from 3-fold with the lower dose of PLZ to 8 to 10-fold with TCP and the high dose of PLZ. The changes in brain catecholamine levels were less pronounced. Tranylcypromine produced the largest and most sustained increase in NE. If one assumes that the rat is a reasonable animal model, all doses used in this study would be considered large (on a mg/kg basis) relative to doses used clinically. It appears from this study that maximum neurochemical changes most likely precede the onset of detectable therapeutic benefit of MAOIs. Tranylcypromine did not affect tyrosine hydroxylase or tryptophan hydroxylase activity.

Two other studies have examined long term effects of selective MAOIs on monoamines. Campbell et al. (1979) reported consistent elevations of NE in rat brain during clorgyline administration while 5-HT levels returned to baseline levels, similar to the effect Robinson et al. (1979) reported for PLZ and TCP. There were no significant changes in levels of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT in the CNS, at any time interval. Dopamine was elevated slightly on day 14, but returned to control levels after 21 days of a 1 mg/kg/day regimen of clorgyline.

Waldmeier et al. (1981) also examined chronic clorgyline treatment in rats and found somewhat different results. Brain NE and 5-HT levels were increased markedly and simultaneously with a concomitant small (nonsignificant) decrease in synthesis. In contrast to Campbell et al. (1979), these workers found that NE and 5-HT levels remained elevated throughout the 21-day regimen, and significant decreases were seen in 5-HIAA. Dopamine levels increased in the cortex and to a lesser extent in the striatum. Large decreases were seen in tyrosine hydroxylase activity in the striatum, which explains why only moderate increases in DA were observed. In the cortex, where DA attained higher levels, there was a much smaller decrease in tyrosine hydroxylase activity, suggesting that feedback inhibition on DA synthesis is less prominent in the cortex compared to the striatum. Recent evidence indicates that mesocortical DA systems lack cell body and nerve terminal DA autoreceptors which are present in nigrostriatal and mesolimbic DA systems (Bannon et al., 1981; Bowers and Salomonsson, 1982). The relationship between DA autoreceptors and tyrosine hydroxylase activity is not known.

Postsynaptic receptor dynamics have also been assessed after chronic MAOI treatment. Savage et al. (1980) demonstrated that chronic (4-16 days), but not acute, doses of TCP, clorgyline, and nialamide significantly reduced binding of ^{3}H-5-HT in rat brain. Scatchard analysis showed a decrease in B_{max} while no change was observed in the K_m. Neither deprenyl nor pargyline affected binding of ^{3}H-5-HT. Surprisingly, a variety of 5-HT reuptake inhibitors, including chlorimipramine, fluoxetine, and amitriptyline had no effect on 5-HT binding. Levels of 5-HT were elevated throughout the dosing period with the MAOIs, while only a transient increase in 5-HT levels was observed with fluoxetine and chlorimipramine. This study suggests that a sustained increase in 5-HT is requisite for a decrease in 5-HT binding.

Chronic regimens of most, if not all, antidepressant treatments decrease β-adrenoceptor-stimulated cAMP (Sulser et al., 1978; Sugrue, 1983). Cohen et al. (1982) have studied the α-adrenergic system in rat after chronic clorgyline treatment. They found that there is a KCl-stimulated increase in NE release from rat brain microsacs which preceded decreases in both α- and β-adrenoceptors. Noradrenergic feedback control, based in part on autoreceptors, is thought to be responsible for the modulation of NE release, both the amount of NE released per impulse, and the readiness for neuronal discharge (Langer, 1977). Cohen et al. (1982) state, "We have proposed that an overly stringent feedback system could impair the capacity of the catecholamine pathways to convey information adequately during depression (Cohen et al., 1980); the present data support the role in potentially resetting this mechanism." This "resetting" would manifest itself as a subsensitization of α-adrenergic (and possibly β-adrenergic) autoreceptor populations.

In summary, it is apparent that monoamine neurotransmitter systems are in a state of flux during antidepressant treatments. It is still equivocal as to whether the initial increase in amines is maintained after chronic treatment. Of critical importance are the facts that (1) these pre- and postsynaptic receptor changes are not apparent after acute treatment; longer term administration is required, and (2) a wide variety of antidepressant treatments, including the so-called first and second generation antidepressant drugs and electroconvulsive therapy, have been demonstrated to induce these changes in β-adrenoceptors. More extensive testing is required of additional drugs and other systems, such as the α-adrenoceptors.

4. Differential Effects of Tranylcypromine Isomers

The clinically used preparation of TCP, called Parnate®, is a racemic mixture of 1R,2S-(-)-trans-phenylcyclopropylamine and 1S,2R-(+)-trans-phenylcyclopropylamine (Riley and Brier, 1972). Horn and Snyder (1972) first examined the effects of (+)- and (-)-isomers on inhibition of reuptake of NE and DA into synaptosomes; they reported that the (-)-isomer was 3 times more potent than the (+)-enantiomer in inhibiting NE reuptake in the hypothalamus and 4 times more potent in inhibiting DA reuptake into striatal synaptosomes. It had previously been shown that the (+)-enantiomer was a more potent inhibitor of MAO, based on its greater ability to potentiate tryptamine convulsions *in vivo* (Zirkle *et al.*, 1962). The greater MAO-inhibiting potency of the (+)-enantiomer was subsequently verified by Fuentes *et al.* (1976), who reported that (+)-TCP was between 10 and 60 times more potent than (-)-TCP, depending on the amine substrate used. These workers also reported that both isomers were better inhibitors of MAO-B than of MAO-A. Smith and Petersen (1982) examined the effects of (+)- and (-)-TCP on brain levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). At doses between 1.5 mg/kg and 30 mg/kg, (+)-TCP caused an increase in 5-HT levels whereas (-)-TCP had no significant effect. Only the high dose of (+)-TCP (30 mg/kg) decreased 5-HIAA levels. These preliminary results demonstrate that there are stereoselective effects of TCP enantiomers on serotoninergic systems.

Reigle *et al.* (1980) studied the effects of TCP isomers on NE metabolism in rat brain. It was found, after intracisternal administration of ^{3}H-NE, that both isomers inhibited its deamination, but the (+)-isomer was considerably more potent. The (-)-isomer was found to enhance the disappearance of endogenous and tritiated NE from the brain. The (+)-isomer at comparable doses was devoid of this effect. Although this action appeared to result from an increase in catecholamine release, the possibility of a contribution from uptake inhibition could not be eliminated. Despite the differential properties of the two isomers, the largest increases in brain levels of ^{3}H-normetanephrine, the O-methylated metabolite of NE, were produced by racemic TCP.

The effects of TCP enantiomers on the behavior of rats pretreated with reserpine have been examined in several studies. This test is widely used as a drug screen for potential antidepressant efficacy despite the fact that its use as a model for depression has been questioned (Fuentes _et al._, 1976). Both Smith (1981) and Fuentes _et al._ (1976) found that the (+)-enantiomer of TCP was more potent than the (-)-enantiomer in antagonizing the sedative effects of reserpine.

A number of studies have reported the effects of TCP isomers in humans. Gorenstein and Gentil (1981) examined the effects of TCP isomers using a battery of tests, both physiological (electrocardiogram, blood pressure, pulse rate, pupil size) and psychological (digit-symbol substitution test, symbol copying test, cancellation task, time estimation, visual analog global impression, mood scale, and bodily symptom scale). In this double-blind study, no significant differences were found between the isomers when compared either to each other or to a placebo. However, side effects, particularly those associated with the sleep-waking cycle, were more consistently reported with the drug groups. The (+)-isomer was associated with more awakenings during the night, while delayed sleep onset was more frequently reported with (-)-TCP when given at night. When administered in the morning, both isomers caused sleepiness that was more frequent and more intense than that with placebo. This is contrary to the stimulating effects of (±)-TCP reported both in clinical studies (Gentil et al., 1978; Himmelhock et al., 1982) and in antagonism of reserpine-induced depression in animals (Smith, 1980).

The first clinical trial comparing TCP isomers (in 11 depressed patients) reported that the (-)-isomer was a more effective antidepressant than the (+)-isomer, and that the latter showed a higher incidence of side effects (Escobar et al., 1974). Moises and Beckman (1981) have criticized this study on the basis of the difference in the global depression scores at the beginning of the clinical trial, the scores being 25 for the (-)-TCP treatment group and 16 for the (+)-TCP group. In their own double-blind, controlled study of TCP isomers, Moises and Beckman (1981) found that statistically significant improvement was apparent only in the (+)-TCP group. They also reported a greater incidence of side effects with (+)-TCP. Their conclusion was that the antidepressant effect was primarily the result of MAO inhibition.

The greater MAO-inhibiting properties of (+)-TCP have also been used advantageously in the treatment of Parkinson's disease (Reynolds et al., 1981). It was found that low doses (3 mg/day), of the (+)-isomer in particular, are effective as an adjuvant to L-dopa therapy. It was claimed that, at the dosages administered, side effects were minimal.

Since studies on TCP isomers have consistently shown them to have stereoselective effects on 5-HT mechanisms, it is of interest to attempt to explain their actions at the molecular level. Smith and Petersen (1982) have pointed out that a common feature of (+)-TCP, lysergic acid diethylamide ([+]-LSD), and L-tryptophan is the spatial relationship between the nitrogen atom in the ethylamine group and the phenyl ring. This suggests that (+)-TCP has the potential to interact directly with 5-HT receptors, as has been shown with LSD (Peroutka and Snyder, 1979), but the situation is obviously much more complex. Further research at the molecular level is necessary to determine why TCP isomers, tryptophan, LSD, and other drugs which interact with 5-HT systems exert such widely differing effects.

In summary, the differences between the TCP isomers have been examined at the chemical, neurochemical, physiological, and behavioural levels. Many of these studies were most likely prompted by the observation of Horn and Snyder (1972) that (-)-TCP was a more potent inhibitor of amine reuptake than (+)-TCP. They also suggested that knowledge of the differential antidepressant efficacy of (+)- and (-)-TCP might indicate the extent to which the drug's antidepressant activity is related to inhibition of amine uptake or inhibition of monoamine oxidase.

The following statements may be made summarizing work since then:

(1) The initial observations of Zirkle et al. (1962) and Horn and Snyder (1972) have been substantiated. That is, the (+)-isomer is a more potent MAO-inhibitor, whereas the (-)-isomer is a more potent inhibitor of the reuptake of catecholamines. However, detailed studies of reuptake inhibition versus amine release have not yet been done.

(2) TCP isomers have definite effects on 5-HT systems, and this property may contribute to clinical efficacy. Again, detailed studies of reuptake versus release dynamics are lacking.

(3) The small number of clinical trials have not determined adequately which, if either, of the TCP isomers is more efficacious as an antidepressant.

(4) Several authors have suggested that knowledge of the differential effects of TCP isomers might be of value in determining the mode of action of TCP and related drugs on endogenous neurotransmitters at the molecular level.

5. Metabolism of Tranylcypromine

The metabolism of TCP has not been extensively studied. Structurally, TCP is similar to amphetamine (AMP), but its neurochemical properties are different. This difference is to a large extent quantitative rather than qualitative because both compounds cause release of biogenic amines and inhibit their reuptake, although AMP is a relatively weak MAO inhibitor. Unlike TCP, AMP is a reversible MAO inhibitor (Clarke, 1980).

In a comparative metabolism study of TCP and AMP, Alleva (1965) determined that 4% of injected ^{14}C-TCP (5 mg/kg) was excreted unchanged, and 12% was excreted as ^{14}C-hippuric acid. The corresponding values for AMP were 15% and 2%. Approximately 71% of both amines were excreted in the urine, mainly as metabolites, within the first 24 hours. The identification of hippuric acid as a metabolite demonstrates that the cyclopropyl ring can be broken; however, it was concluded that this cleavage does not involve the formation of AMP, based on different patterns of peaks in the paper chromatograms obtained in this study. It was suggested that a liver microsomal enzyme system described by Axelrod (1955) which deaminates α-alkylamines to ketones may be involved in the ring-splitting reaction.

Contrary to the animal study by Alleva, Youdim _et al._ (1979) reported the detection of AMP, methamphetamine, and PEA in the plasma of a TCP overdose victim. In this report, analyses were done by GC and GC-MS.

Theoretically, TCP could also be metabolized to _para_-hydroxy-TCP (_p_-OH-TCP). _Para_-hydroxyamphetamine (_p_-OH-AMP) has been detected as a metabolite of AMP _in vivo_ and _in vitro_ in the rat (Axelrod, 1954; Billings _et al._, 1978; Prelusky, 1983). The enzyme system responsible for this reaction has not been fully characterized. Results of studies done by Billings _et al._ (1978) indicate that the hydroxylation system has most of the properties of the cytochrome P-450 mixed-function oxidase (MFO) system found in microsomes. It is inhibited by CO and by SKF-525A and is particularly sensitive to the inhibitory effects of the antidepressant iprindole. It also has a dependence upon NADPH. However, unlike other substrates of the P-450 system, the AMP hydroxylating system is not induced by phenobarbital or 3-methylcholanthrene.

Tranylcypromine is an inhibitor of the microsomal MFO system by its interaction with cytochrome P-450 (Belanger and Atitse-Gbeassor, 1982). This MAOI is a competitive inhibitor of N- and O-demethylations of aminopyrine and p-nitroanisole respectively, but is a non-competitive inhibitor of the N-demethylation of N,N-dimethylaniline and of aniline hydroxylation. Tranylcypromine gives a typical type II difference spectrum with a half-maximum change value (K_s) of 0.3 mM. These results show that TCP is more strongly bound to cytochrome P-450 than are aminopyrine and aniline since their K_s values are 1.8 and 2.6 mM respectively. In addition, preincubation of TCP for 30 min. with fortified microsomal homogenate, prior to addition of substrate (aniline), resulted in a decrease of the inhibitory effect of approximately 50% as estimated by comparison of Ki values obtained with and without preincubation (Belanger and Atitse-Gbeassor, 1982). It can therefore be concluded that TCP itself is responsible for the inhibitory effect on cytochrome P-450 and that the metabolic products, although unidentified in this study, contribute little, if at all, to the inhibition of this enzyme system.

Although p-OH-TCP may not contribute to inhibition of the MFO system, it may have other active neurochemical properties. Analogous parahydroxylated metabolites have been shown to possess biological activity. For example, p-OH-AMP has been shown to produce a pressor effect (Maxwell et al., 1959) and to be taken up into synaptosomal preparations from rat striatum and cortex (Cho et al., 1977). In addition, p-OH-AMP appears to be approximately equipotent to AMP in inhibiting the reuptake of NE and in causing release of this neurotransmitter (Wenger and Rutledge, 1974). Wenger and Rutledge (1974) also determined that parahydroxynorephedrine, another metabolite of AMP, was substantially less active than AMP or p-OH-AMP in causing release or inhibition of reuptake of NE.

Para-hydroxylation of another related compound, PEA, to form paratyramine (p-TA) has also been shown to potentiate the release of NE, DA, and 5-HT from synaptosomes and to increase the inhibition of reuptake of these amines into synaptosomes (Raiteri et al., 1977).

In summary, a potentially important metabolic pathway for TCP is oxidation by the microsomal MFO system. In contrast to AMP, which appears to be a substrate for this system, albeit somewhat atypical, TCP is an inhibitor. The consequence of being an inhibitor of both the MFO system and MAO is not fully understood. Although TCP may inhibit its own metabolism to p-OH-TCP, any p-OH-TCP formed may contribute to the effects of TCP on release and/or inhibition of reuptake of aminergic neurotransmitters. The hypothetical pathways for the metabolism of TCP are depicted in Figure 3.

6. Formation of Tetrahydro-β-Carbolines after the Administration of Tranylcypromine and 5-Hydroxytryptophan

Tetrahydro-β-carbolines (THBCs) are the condensation products of indoleamines with aldehydes via the Pictet-Spengler reaction (Figure 4). Some of these THBCs form readily under physiological conditions (Whaley and Govindachari, 1951). In recent years, interest in them has grown not only because they have been detected as normal constituents of body fluids of animals and humans, but also because they appear to have a variety of biological effects (see Airaksinen and Kari, 1981, Buckholtz, 1980, and Bloom *et al.*, 1982 for reviews).

Structurally, THBCs are related to the harmala alkaloids, specifically the aromatic β-carbolines harmaline, harmine, and harmalol. These compounds are found in the Amazonian plants of the genus *Banisteriopsis* and in the Middle East in the plant *Peganum harmala*. These and some of the THBC congeners have been found to be highly hallucinogenic and have been used for the purpose of inducing such states by the native peoples where the plant sources are found (Naranjo, 1967). As with most other hallucinogenic drugs, their exact mode of action is unknown, although a variety of biochemical and neurochemical actions have been described.

It is well known that THBCs inhibit MAO (Buckholtz, 1980). It appears that they are better inhibitors of MAO-A than MAO-B, both *in vivo* and *in vitro*. Meller *et al.* (1977) reported the IC_{50}s (μM) for THBC, 6-methoxy-THBC, and 6-OH-THBC as 3.7, 3.7, and 28 respectively for

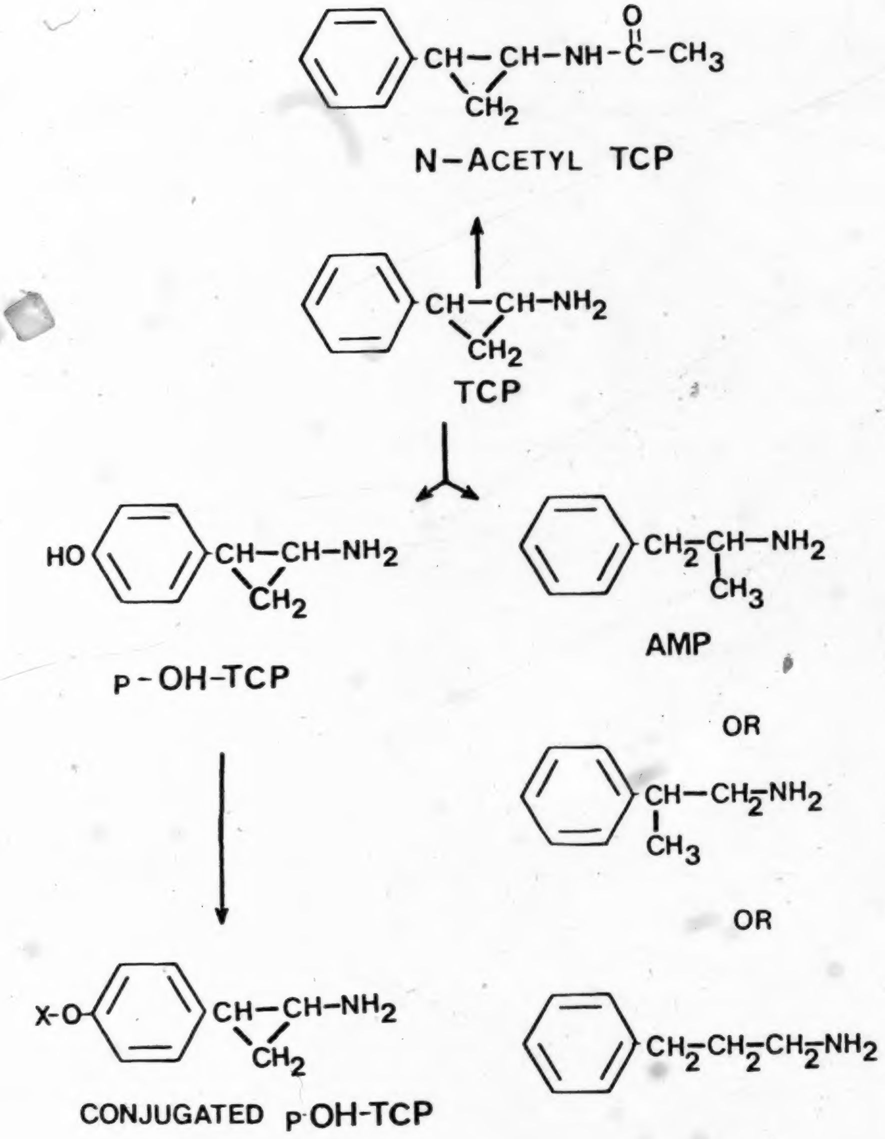

N-ACETYL TCP
TCP
p-OH-TCP
AMP
OR
OR
CONJUGATED p-OH-TCP
X-O
HO

Figure 3. Proposed metabolic pathways for tranylcypromine.

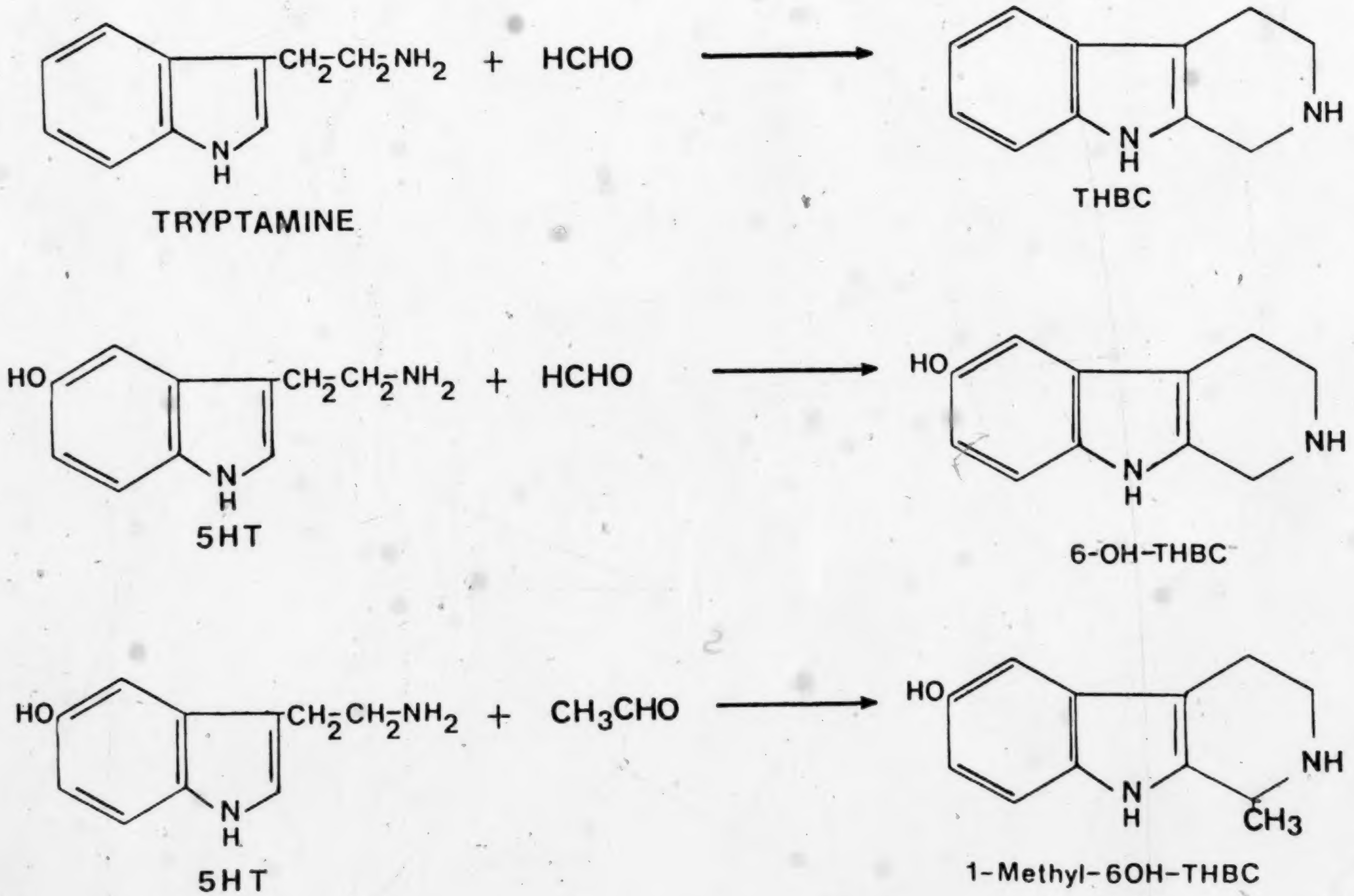

TRYPTAMINE
HCHO
THBC
5HT
HCHO
6-OH-THBC
5HT
CH3CHO
1-Methyl-6OH-THBC

Figure 4. Condensation of aldehydes with indoleamines to form tetrahydro-β-carbolines (THBCs) via the Pictet-Spengler reaction.

5-HT as substrate, and 250, > 1000, > 1000 for PEA as substrate. The β-carboline harmaline is a reversible MAO-A inhibitor and is, in this respect, similar to AMP (Green, 1980).

THBCs also inhibit the reuptake of 5-HT into synaptosomes. Friedman et al. (1980) have reported IC_{50}s of 0.86, 0.54, and 0.13 μM for THBC, 6-methoxy-THBC, and 6-OH-THBC respectively. Therefore these β-carbolines are more potent inhibitors of 5-HT reuptake than inhibitors of MAO. The THBCs are more potent than the corresponding aromatic β-carbolines in inhibiting the reuptake of 5-HT (Buckholtz and Boggan, 1977).

Another way in which THBCs could affect 5-HT metabolism would be by a modification in neurotransmitter release. The published data on the effects of THBCs on 5-HT release are somewhat contradictory. Kellar et al. (1976) found no effect of 6-OH-THBC (0.4 μM) on release of radiolabelled 5-HT from synaptosomes. In slices of bovine retina, Thomas et al. (1979) found that 6-methoxy-THBC (1 μM) increased the Ca^{++}-dependent K^{+}-stimulated release of 5-HT by about 50%. In evaluating these studies, it appears that in the hypothalamus there does seem to be increased release of 5-HT by THBC and 6-OH-THBC, but the per cent release is considerably less than the per cent inhibition of reuptake at comparable concentrations.

Because of the structural similarities between 5-HT and the THBCs, the THBCs may themselves be taken up into 5-HT neurons where they could act as neuromodulators. The data on inhibition of MAO after administration of 6-methoxy-THBC (Buckholtz and Boggan, 1977) suggest that it does enter nerve endings since most MAO is located on the outer mitochondrial membrane. Also the selective effects of THBCs on increasing 5-HT but not catecholamine levels could be the result of preferential uptake into 5-HT-containing neurons (Buckholtz, 1980).

All the effects mentioned above are presynaptic neurochemical actions. Cascio and Kellar (1982) have examined the affinities of THBCs for 5-HT and T receptors. They reported that THBC and 6-OH-THBC have relatively low affinity for 5-HT_1 and 5-HT_2 binding sites in rat cerebral cortex, suggesting that presynaptic effects may be more important than postsynaptic effects on 5-HT systems.

Aside from the direct and indirect actions of THBCs on neurotransmitter systems, particularly the 5-HT system, interest in these compounds has also been boosted by the discovery that β-carboline-3-carboxylic acid ethyl ester is a potent inhibitor of binding of benzodiazepines (BZD) at their receptor site (Braestrup and Nielsen, 1980). Although this compound was isolated from human urine, it appears that the ester function is an artifact of the isolation procedure itself. The free acid has an affinity of about 4500x less than the ethyl ester (IC_{50} of 31 μM vs 7 nM for the ethyl ester) but has not been detected _in vivo_. Harmane (1-methyl-β-carboline) appears to be the most potent endogenously-occurring inhibitor of BZD binding yet found, but its IC_{50} is also rather low (IC_{50}; 7 μM) (Rommelspacher _et al._, 1980).

Nevertheless, much work and even more theoretical speculation on the possibility of an endogenous ligand for the BZD receptor analogous to those for the endogenous opiates has been initiated by this discovery. Recent reports (Ninan _et al._, 1982 and Dorow, 1982) indicate that several of the carboxylic acid derivatives of β-carbolines may be anxiogenic in humans and monkeys. This would suggest that the endogenous ligand, if it exists, may be anxiogenic. However, small changes in the β-carboline structure drastically alter the agonist-antagonist properties of these compounds (Martin, 1980), so that a much more complex modulation of the system may be possible.

Another area of active research on THBCs is their formation after administration of substances which may result in increased levels of THBCs. Formation of THBCs after ethanol administration has been studied by a number of groups. In vivo, ethanol is metabolized to acetaldehyde which can condense with indoles to form THBCs. Rommelspacher et al. (1980) reported detecting 1-methyl-THBC and 1-methyl-β-carboline in the urine of rats and humans after ethanol administration. Peura et al. (1980) detected 1-methyl-THBC in human blood after ingestion of ethanol.

A major impetus in research on the formation of β-carbolines after ethanol ingestion is the possibility that these compounds may be involved in alcohol addiction (Holman et al., 1980). Behavioral studies in this area have produced contradictory results. Messiha et al. (1977) have reported that chronic administration of 6-methoxy-tetrahydroharman or acute administration of 6-methoxy-THBC, 1-methyl-THBC, or THBC reduced ethanol consumption in rats. Myers and Melchoir (1977) found that intraventricular administration of THBC (either chronic infusion every 30 min for 12 days or injection once/day for 12 days) significantly increased alcohol ingestion in rats. Certain symptoms of withdrawal were seen during the course of chronic administration. Further complicating the situation is a study by Beck et al. (1982) who reported that there was no relationship between ethanol intake and the levels of 1-methyl-THBC or 6-OH-1-methyl-THBC in rats or humans. In fact, both of these compounds were reported to be present in human urine from control subjects. Subsequent studies by this group showed that these compounds were present in a variety of foods, including beer, wine, cheese, and bananas. The consumption of 5 bananas by two subjects greatly increased their urinary excretion of 6-OH-1-methyl-THBC. Although diet may be one source of THBCs in humans, THBCs were still detected in rat urine despite the inability to detect these compounds in rat food.

Theoretically, another way to increase formation of THBCs is to increase concentrations of indoles. In the study by Beck et al. (1982), some of the foods containing high THBC concentration also contain considerable amounts of tryptophan (e.g. bananas). Tryptophan, the dietary precursor of 5-HT, has been used as an antidepressant and an antimanic agent (van Praag, 1981). Tryptophan loading increases 5-HT and T levels in the brain (Warsh et al., 1979). Rommelspacher et al. (1979) have demonstrated that 6-OH-THBC could be detected in human urine after a dose of 5-hydroxytryptophan, the immediate precursor of 5-HT. This compound could not be detected in urine in untreated subjects, although it was detected in platelets of untreated subjects.

Another way to increase 5-HT and T levels is by the administration of MAOIs. The formation of THBCs after treatment with MAOIs has not been reported. The combination of an MAOI with tryptophan has also been used on a limited basis as an antidepressant therapy (Glassman and Platman, 1969; Alino et al., 1976). This drug regimen, when administered to rodents, produces a bizarre behavioral syndrome characterized by stereotypy, headweaving, forepaw treading, hindlimb extension, salivation, and straub tail (Hess and Doepfner, 1961; Hampson, personal observation). It has been shown that the concentrations of 5-HT and T rise dramatically after injection of this drug combination to rats (Hay et al., 1982). Insel et al. (1982) described two patients given a single dose of the tricyclic antidepressant clomipramine who apparently displayed the 5-HT syndrome. This response may have resulted from an interaction with clorgyline (MAOI) given to the patients several weeks earlier.

The involvement of the β-carbolines, if any, in the 5-HT syndrome is not known, although optimal conditions may be present for their formation under these conditions. Two of the most likely THBCs to be formed would be the condensation products of 5-HT and T with formaldehyde, to form 6-OH-THBC and THBC respectively.

A major problem encountered in studies on detection of THBCs is the difficulties involved in their analysis. Their poor solubility, relatively high molecular weight, and presence in trace quantities all contribute to the difficulties associated with their analysis. In addition, the amphoteric nature of 6-OH-THBC further contributes to the problems involved with extraction and isolation. Bosin et al. (1982) have outlined many of the analytical pitfalls encountered in the identification and quantitation of endogenous THBCs. Of major concern was the artifactual formation of THBCs after sample collection. Trace quantities of aldehydes in the solvents and reagents used or in the tissue samples themselves posed a formidable problem. This was circumvented by using pure "aldehyde-free" solvents, aldehyde-trapping agents (semicarbazide, 5-methoxytryptamine), and short extraction and workup times. Under these conditions, reproducibility was greatly improved.

To summarize, the β-carbolines and THBCs have a wide spectrum of neurochemical actions. It appears that interactions with 5-HT at the presynaptic level are the most prominent. They have been hypothesized to be involved with such diverse phenomena as anxiety and stress to alcohol addiction. Several analogs of the β-carbolines are potent hallucinogens; however, the behavioral effects in humans of many of these compounds is unknown. A number of studies have detected several THBCs as normal constituents of body tissues and increased levels after various drug treatments. A fundamental problem has been the accurate analysis of these compounds due to a variety of analytical difficulties and such problems have been the target of several valid criticisms. Due to recent advances in analytical techniques, future research may obviate these problems.

7. Outline of Research Programme

(a) Acute and Chronic Administration of Tranylcypromine

Although the acute effects of TCP on monoamine systems have been known for some time, the effects of long-term treatment have not been thoroughly investigated. Current theories on the mode of action of antidepressants are evolving towards the study of various biological parameters after long-term treatments. This has been instigated primarily by the temporal discrepancy between acute neurochemical effects, which take place immediately, and the remission of the symptoms of depression, which are usually delayed for 1 to 4 weeks.

Both pre- and post-synaptic neurotransmitter mechanisms have been examined after long-term antidepressant treatment, but the few studies available have produced contradictory results. This is particularly true of the presynaptic components of neurotransmitter dynamics involved with synthesis and regulation of the neurotransmitters and hence their levels in the body.

As stated above, reports of both sustained increases in amine levels (Waldmeier et al., 1981) and a return to baseline values (Robinson et al., 1978; Murphy et al., 1978) have been reported with chronic MAOI treatment. In addition, in most of these studies, extremely high and clinically irrevelant doses of drugs have been used. The use of a therapeutic dose of an antidepressant during a chronic study is paramount to obtaining meaningful results since the dose may be directly associated with the time element involved with changes of neurotransmitter levels and the maximum response obtained. Many studies also suffer from a lack of comprehensiveness. For example, during long-term MAOI administration, reports of neurotransmitter levels without concomitant measurement of MAO inhibition and/or drug levels have been the status quo. Other criticisms include short duration of drug administration and the measurement of only one or two amines.

The present study has attempted to remedy these deficiencies by conducting a more comprehensive neurochemical study of the effects of long-term treatment with TCP at a low, clinically-relevant dose. Rats were injected daily with 1 mg/kg TCP for up to 42 days. At various time periods during TCP administration, rats were either sacrificed and brains removed for analysis, or urine was collected for analysis.

Brain tissue was analysed for biogenic amines from 3 major classes of neurotransmitters: the catecholamine DA, the indolealkylamine 5-HT, and the trace amines T and PEA. All of these amines have been proposed as being involved in various psychiatric disorders. The amines selected include the MAO-A substrate 5-HT, the MAO-B substrate PEA, and the proposed mixed substrates DA and T. Urine was analysed for 5-HT, T, PEA, and the dopamine metabolite 3-methoxytyramine (3-MT). 3-Methoxytyramine rather than DA was analysed due to the instability (oxidation) of DA in solution. Inhibition of MAO-A and MAO-B was measured in vivo in all brain samples analysed.

Finally, an extremely sensitive and reproducible method for the analysis of TCP in brain tissue was developed. Such a method was required to measure TCP in brain tissue because of the low dose used.

(b) Administration of Tranylcypromine Isomers

Although the acute effects of TCP on some of the amine systems have been examined, few studies have looked at the effects of the separate optical isomers of TCP on brain amine levels. Some researchers have studied the actions of the isomers on inhibition of brain MAO-A and MAO-B in vivo, but in none of these investigations have the actual brain concentrations of the amines which are specific substrates for these two forms of MAO (5-HT and PEA respectively) been reported. Previous studies have investigated the potency of TCP isomers on MAO inhibition (Zirkle et al., 1962) and amine reuptake inhibition (Horn and Snyder, 1972), but these studies have utilized relatively crude assay techniques (e.g. Zirkle et al., 1962 measured MAO inhibition by potentiation of T-induced convulsions) or techniques which may have produced physiologically irrelevant results (Horn and Snyder, 1972; see Section IV.B.3 for further discussion).

The present study measured the effects of TCP on several neurochemical parameters after the administration of 1 mg/kg of either the (+)- or (-)-isomer. The biogenic amines 5-HT and PEA (substrates for MAO-A and MAO-B) were measured in the brain at 0.5, 1.5, 3.0, and 6.0 hr after drug administration. Inhibition of MAO-A and MAO-B was measured *in vivo* for both isomers, and inhibition of MAO was also determined *in vitro*. The combination of *in vivo* and *in vitro* experiments was expected to produce a more accurate indication of the relative potency of the isomers.

In addition, the inhibition of reuptake of ^{3}H-NE in the hypothalamus and of ^{3}H-DA and ^{3}H-5-HT in the striatum *in vitro* by (+)- and (-)-TCP was also determined. In another series of *in vitro* experiments, the ability of (+)- and (-)-TCP to release these tritiated neurotransmitter amines from brain tissue was examined. There have been no reports in the literature that have looked at release of amine neurotransmitters by the TCP isomers using a method which measures "pure" release (Raiteri *et al.*, 1974). The effects on release of ^{3}H-NE from the hypothalamus and ^{3}H-DA and ^{3}H-5-HT from the striatum were investigated.

It is hoped that the results of these studies will give a better understanding of the response of several neurotransmitter systems to the two stereoisomeric forms which make up the TCP used commercially. Results of these studies could give further insight into the relative sensitivity and flexibility of various enzyme and uptake systems in neurons.

(c) Detection and Characterization of Para-Hydroxytranylcypromine

A number of compounds structurally related to TCP, particularly AMP, have been shown to be *para*-hydroxylated and it has been demonstrated that these oxidized metabolites are biologically active. Only one major metabolic study of TCP has been reported in the literature (Alleva, 1965) and this study was both incomplete (verification of only 2 metabolites) and relatively crude (paper chromatography was used for separation and structural determination).

The present study has utilized gas chromatography (GC) and combined GC-mass spectrometry (MS) for detection of p-OH-TCP. The sensitivity and selectivity of the combination of high-resolution glass capillary GC columns and that inherent in the mass spectrum itself are considered by many to be the best technology available for drug metabolism studies which attempt to detect, quantitate, and characterize minute quantities of drug metabolites in body fluids. Since no methods for the analysis of p-OH-TCP have been reported in the literature, methods were developed for its analysis in both brain tissue and urine. For analysis of p-OH-TCP in brain, a method with very low limits of detection was required since it was anticipated that low levels of p-OH-TCP may be present in the brain.

Studies were also undertaken to determine whether p-OH-TCP was a biologically active metabolite. Monoamine oxidase inhibition was studied *in vitro* along with determination of the neurotransmitter reuptake inhibiting and releasing properties of p-OH-TCP.

Another important aspect of this study was the determination of the relative influence of pretreatment with various drugs on *para*-hydroxylation of TCP. Although these methods are indirect in that levels of the parent drug TCP rather than the metabolite are measured, such information helps to substantiate the existence and extent of ring hydroxylation. These experiments also have clinical relevance since some of these drug combinations are used by some physicians (the TCP + trifluperazine combination is a commercial pharmaceutical product). These experiments may also be of value in determining the potential for neurotoxicity. Fuller and Hemrick-Luecke (1980) have shown that in iprindole-pretreated rats AMP causes a long-lasting depletion of DA in the CNS. They suggested that since AMP is a well-known drug of abuse, the recognition that a single dose of AMP can cause a long-lasting, possibly neurotoxic change in the brain may be of value in exploring a neurochemical basis for persistent behavioral changes in chronic AMP abusers. Although the behavioral effects of chronic low-dose TCP are different from the behavioral effects of chronic high-dose AMP, other similarities between the two drugs exist (e.g. neurochemical actions, use on a continuous basis, and chemical structure). On this basis, the possibility of a neurotoxic reaction is not entirely remote.

(d) Detection of 6-Hydroxytetrahydro-β-Carboline

The literature reviewed in Section I.C.6 reveals that, at the present time, there is widespread interest in β-carboline-related compounds for a variety of reasons, including their structural similarities to hallucinogenic compounds (harmala alkaloids), their diverse neurochemical properties, and their possible involvement with the BZD receptor. Unfortunately, despite the wide interest in this group of compounds, their mere existence *in vivo* has been difficult to prove. Knowledge of the difficulties encountered in their analysis has evolved in the past 5 years to a point where meaningful attempts to detect them *in vivo* are more feasible.

In light of this, an attempt was made to detect and, if possible, quantify THBC and 6-OH-THBC. These THBCs were chosen as the focus of this part of the study because 6-OH-THBC and THBC, respectively, are the condensation products of formaldehyde with 5-HT and T, two indoles that occur naturally in appreciable amounts either normally (5-HT) or after inhibition of MAO (T).

Methods of analyzing THBCs have recently been published (Barker et al., 1981), and initially attempts were made to utilize these procedures. However, due to inaccessibility of some equipment and reagents (e.g. deuterated THBCs for use as internal standards) and the laborious procedures involved (with the possibility of concomitant loss of sample), modification and development of other methods were pursued.

The treatment of rats with TCP and 5-hydroxytryptophan was chosen because:

(1) the administration of an MAOI and tryptophan or 5-hydroxytryptophan has been shown to produce the so-called 5-HT hyperactivity syndrome; therefore the possibility exists that THBCs may play a role in this syndrome; and

(2) this drug combination has been reported to be an effective antidepressant treatment (Glassman and Platman, 1969; Alino et al., 1976).

In addition, the demonstration of THBC formation in the rat would provide preliminary evidence for the use of the rat as a suitable animal model for future studies in this area.

?

MATERIALS AND METHODS

II. MATERIALS AND METHODS

II.A. Animals

All animals used throughout these studies were male Sprague-Dawley rats obtained from Bio Science Animal Services (Ellerslie, Alberta). All rats weighed between 175-250 g, except for rats used in the chronic studies which weighed approximately 300 g at the end of 21 days. Rats were housed in groups of four (except during urine collections when they were housed in individual metabolism cages) on a 12-hour light/12-hour dark schedule. The temperature in the animal care facility was kept at 21° (all temperatures reported are in degrees centrigrade). Rats were fed Lab-Blox (24% crude protein, 4.0% crude fat, 4.5% crude fiber; Wayne Feed Division, Continental Grain Company, Chicago, Illinois) *ad libitum*. Water was also available *ad libitum*.

II.B. Dosing Schedules

1. Acute and Chronic Dosing with Tranylcypromine

Rats were weighed and given a single daily intraperitoneal (i.p.) injection of 1.28 mg/kg TCP HCl (equivalent to 1 mg/kg free base). The drug was dissolved in physiological saline solution. Animals were weighed every second day during chronic treatment. Injections were administered at approximately 3 p.m. All rats were sacrificed by cervical dislocation 1.5 hr after the last dose. Brains were dissected out (pineal gland removed) and immediately placed in ice-cold saline solution. Brains were frozen at -50° within 5 min. On appropriate days, some rats were transferred to metabolism cages for urine collection. While in the metabolism cages, food and water were provided *ad libitum*.

Drug injections were administered immediately prior to the 24 hr urine collection period. Two hundred µl of 2% disodium ethylenediaminetetraacetic acid (EDTA) were added to the urine collection vessels. At the end of the 24 hr urine collection period, urine was transferred to screw cap vials and frozen at -50° until analysis.

2. Tranylcypromine Isomer Studies

Rats were administered a single i.p. injection of 1.28 mg/kg (+)- or (-)-TCP HCl dissolved in a solution of physiological saline. Animals were sacrificed at 0.5, 1.5, 3.0, and 6.0 hr after injection. Whole brains were dissected out (pineal gland discarded), placed in ice-cold physiological saline solution, and frozen within 5 min. at -50° until the time of analysis. Timing of injection was such that all rats were sacrificed between 4:30-5:30 p.m.

3. Metabolism Studies

Rats were injected i.p. with 1.28 mg/kg TCP HCl in physiological saline solution and sacrificed 1.5 hr later; brains were dissected and treated as described above. Other rats dosed in a similar manner were placed in metabolism cages for 24 hr urine collections. Urines were treated as described previously.

For studies on the effects of pretreatment of various drugs on TCP metabolism, groups of rats were injected i.p. with pretreatment drug or saline at 3:00 p.m. All pretreatment drugs were administered at a dose equivalent to 35 µmoles/kg (free base). At 4:00 p.m., these rats were injected i.p. with TCP (1 mg/kg) or AMP (1 mg/kg) and sacrificed 1.5 hr later (5:30 p.m.). The brains were quickly dissected out and frozen at

-50° until analysis.

4. Tranylcypromine/5-Hydroxytryptophan Combination

Rats were administered a 6.4 mg/kg i.p. dose of TCP HCl (equivalent to 5 mg/kg free base) in physiological saline. Thirty minutes later a 15 mg/kg i.p. dose of 5-hydroxytryptophan methyl ester was administered. One hour later, animals were sacrificed and brains treated as described previously.

Some animals were also dosed as above, and then placed in metabolism cages for urine collection. To the urine collection vessels, 200 µl of 2% EDTA and 200 µl of a 250 mg/ l solution of semicarbazide (an aldehyde trapping agent) were added. After 24 hr, urines were transferred to screw-cap vials and frozen at -50° until analysis.

II.C. Reagents

A list of derivatizing reagents used for gas chromatography and gas chromatography-mass spectrometry is shown in Table III. All derivatizing reagents were kept at 4° or below until use. Derivatizing reagents were used in the fume hoods in all analytical experiments.

A list of individual drugs and chemicals used, including the manufacturer, is shown in Table IV.

Organic solvents were glass-distilled and obtained from Caledon Laboratories Ltd. or Fisher Scientific Company. Water was double-distilled in a Corning AG-3 still. Other miscellaneous solid chemicals in reagent grade were obtained from North American Scientific and Chemical Company, J. T. Baker Chemical Company, or Fisher Scientific Company. Acids were purchased from J. T. Baker Chemical Company or Fisher

Table III. A list of derivatizing reagents used for gas chromatography and gas chromatography-mass spectrometry, including manufacturer.

Acetic anhydride (AA), Pierce Chemical Co.

Heptafluorobutyric anhydride (HFBA), Pierce Chemical Co.

Heptafluorobutyrylimidazole (HFBI), Pierce Chemical Co.

Pentafluoropropionic anhydride (PFPA), Pierce Chemical Co.

Pentafluorobenzoyl chloride (PFBZ), Aldrich Chemical Co.

Trichloroacetic anhydride (TCAA), Pfaltz and Bauer, Inc.

Trifluoroacetic anhydride (TFAA), Sigma

Table IV. A list of chemicals and drugs used, including the abbreviation (if any) and the manufacturer.

(±)-Amphetamine (AMP) sulfate (Smith, Kline and French, SKF)

p-Chlorophenylethylamine (p-Cl-PEA), HCl UAFPPS[a]
Chlorpromazine HCl, Sigma
Dopamine (DA) HCl, Sigma
Ethylenediaminetetraacetic acid (EDTA), J. T. Baker
Glyoxylic acid, Sigma
Iprindole HCl, Wyeth Laboratories
p-Hydroxytranylcypromine (p-OH-TCP) HCl, UAFFPS
5-Hydroxytryptamine Creatinine Sulfate (5-HT), Sigma
6-Hydroxytetrahydro-β-carboline HCl UAFFPS[a], DRH[b]
3-Methyoxytyramine (3-MT) HCl, Sigma
5-Methyltryptamine (5-MT) HCl, Sigma

N-Methyldopamine HCl, Sigma
Norepinephrine (NE) (Arterenol) HCl, Sigma
β-Phenylethylamine (PEA) HCl, Sigma
Semicarbazide HCl, Sigma
SKF 525-A, SKF

Trans-2-Phenylcyclopropylamine (tranylcypromine; TCP) HCl, Sigma
Tranylcypromine Isomers, SKF
L-Tryptophan Methyl Ester HCl, Sigma
Trifluperazine HCl, SKF
Tryptamine (T) HCl, Sigma

[a] Obtained as free base from Sigma Chemical Co. and the HCl salt synthesized by Dr. Eric Hall and Dr. Ronald Micetich, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta (UAFPPS).

[b] Author

Scientific Company and diluted with double-distilled water for use.

Acidic aluminum oxide was obtained from Terochem Laboratories. Glass fiber filters (25 mm) were purchased from Schleicher and Schuell, Inc. Paper filters were purchased from Whatman Ltd.

Beta-glucuronidase/aryl sulfatase (*Helix pomatia*) was obtained from Calbiochem-Behring Corporation. Di(2-ethylhexyl)phosphate (DEHPA) was purchased from Sigma Chemical Company. Radioactive substrates including ^{3}H-DA, ^{3}H-NE, ^{3}H-5-HT, ^{14}C-5-HT, and ^{14}C-PEA were obtained from New England Nuclear.

All buffers and scintillation fluid were mixed as needed. The formulas for both are listed in Appendix II.

II.D. Instrumentation

1. Analysis of Biogenic Amines - General Considerations

Various analytical methods have been employed to detect and quantitate biogenic amines in tissues and body fluids (Baker and Coutts, 1982). These include biological assays, thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC), histochemical methods, radioenzymatic procedures, gas chromatography (GC), and gas chromatography/mass spectrometry (MS). Each of these methods has its own inherent advantages and disadvantages.

Of primary concern in the present study was the necessity of utilizing methods with a high level of sensitivity and selectivity. The requirement for a method with a high degree of sensitivity is obligatory when analysis of compounds such as the trace amines are carried out in complex biological tissue such as the brain or in urine where numerous metabolic endproducts are present. This is also true of the detection and quantitation of TCP itself, since this study has utilized low doses of TCP throughout most experiments. For these reasons, together with the need for high selectivity, GC and GC/MS methods were chosen since they fulfill both these requirements.

A GC equipped with an electron-capture detector (ECD) provides an instrument capable of detecting extremely minute quantities (< 10 pg on column) of materials, providing they can capture electrons. In the ECD, thermal electrons are produced by collision of electrons emitted from a radioactive source (^{63}Ni) with carrier gas molecules. The electrons are collected at the anode of the detector cell and produce a standing current. Electron-capturing compounds such as halogens attract and combine with these electrons, which in turn results in reduction of the standing current. It is this minute reduction in current which is actually measured in an ECD. Since most neurotransmitters and other biogenic amines normally absorb electrons poorly or not at all, they must be chemically modified to produce an ECD-sensitive derivative. The derivatives so produced also usually display increased volatility, a feature that is not only mandatory for use in GC, but may also increase selectivity.

The need for high sensitivity was also a factor in the selection of derivatizing reagents chosen. For example, in the development of assays for TCP (Hampson _et al._, 1984a) and PEA (Hampson _et al._, 1984b), PFBZ was the reagent of choice because (a) it possesses an extremely high capacity to capture electrons and hence is one of the most ECD-sensitive derivatizing agents known (Matin and Rowland, 1972; Bock and Waser, 1981); (b) it is capable of reacting with phenolic-OH groups, primary amines, and some secondary amines under anhydrous or aqueous conditions. The ability to react under aqueous conditions is particularly desirable, since a so-called phase transfer reaction may be employed to extract the derivatized biogenic amines from aqueous media such as urine or supernatants from tissue homogenates; and finally, (c) PFBZ derivatives are usually stable for long periods of time when stored at temperatures of 4° or lower. Unfortunately, PFBZ cannot be used in all cases; for example, some secondary amines and tertiary amines do not react with PFBZ, and in other cases the PFBZ derivative may coelute on GC with endogenous interfering substances present in an extract. In some of these cases, other derivatizing reagents (Table III) have proved valuable.

Although a GC equipped with an electron-capture detector and a capillary column does provide a sensitive, rapid and inexpensive (relative to GC-MS) method for routine analysis of many amines in tissues and body fluids, it is still necessary to confirm structures of all derivatives by GC-MS when developing new analytical techniques. In addition, it has also been observed that even with the use of high-resolution capillary columns, inadequate GC selectivity is encountered in some instances, i.e. the derivative of interest does not separate on the GC column from an interfering peak. This may also occur on a GC-MS system; however, interferences would become apparent in the mass spectrum of the peak in question and by careful selection of fragment ions unique to the compound of interest, single or selected ion monitoring (SIM) could be utilized to provide a specific analysis. Also, the choice of electron-impact (EI) or chemical ionization (CI) MS gives further versatility to a GC-MS system. An EI mass spectrum usually provides numerous fragment ions which are of value in structural determinations, whereas in CI-MS, less fragmentation occurs (due to the "softer" ionization inherent in

CI-MS) and a spectrum which contains an abundant quasimolecular (MH^+) ion usually results. An abundant quasimolecular ion is extremely useful for the determination of molecular weight and for use in SIM quantitative studies.

From the information presented above, it is apparent that GC-ECD and GC-MS are two techniques which, when used in conjunction with each other, provide a powerful analytical tool. The major limiting factor in their use in neurotransmitter analysis is the necessity of forming a volatile derivative. Although for the experiments conducted in this study, this restriction was generally overcome, other analyses, especially those which involve molecules with a large number of functional groups, may be better suited for other techniques such as HPLC or RIA, which usually do not require volatile derivatives.

2. Gas-Liquid Chromatography

All GC was performed on one of the following instruments: a Hewlett Packard (HP) 5880 or an HP 5880A gas chromatograph. The instruments were equipped with an Ni-63 electron-capture detector and either a 12 m OV-101 or OV-1 fused silica capillary column. Operating conditions for these two instruments were: carrier gas (He), 20 pounds per square inch (p.s.i.); make up gas (argon/methane 90/10) at a flow rate of 35 ml/min.; injection port temperature 270°; detector temperature 300°. Some analyses were also performed on an HP 5830A gas chromatograph equipped with a 2 m glass column (4 mm i.d.) packed with 3% OV-1 on Gas Chrom Q. The carrier gas was argon/methane (90/10) at a flow rate of 40-60 ml/min. The detector temperature was 300° and the injection port temperature was 270°. All quantitations were based on peak heights using an internal standard.

3. Mass Spectrometry

Electron impact, CI, and SIM mass spectrometry were performed on an HP 5985A quadrapole mass spectrometer equipped with an HP 2648A graphics terminal, HP 9876A printer, HP 7920 disc drive (software), and HP 21MX Series E computer (hardware). Inlet mode was via gas chromatography, specifically a 12 m wide-bore SE-54 fused silica column. Standard GC-MS conditions were as follows: oven conditions, 90° for 0.6 min., increasing at a rate of 30°/min. to a final temperature of 275° which was maintained for 10 min.; injection port temperature, 280°; interface temperature, 275°; ion source temperature, 200°; column pressure, 10 p.s.i.; methane (CI) pressure, 3 x 10^{-4} Torr; accelerating voltage (EI), 2200 eV; accelerating voltage (CI), 2600 eV; ionization voltage, 70 eV; scan speed, 100 amu/sec.; dwell time, 200 msec. (total ion scan), 500 msec. (SIM).

Data retrieval and analysis were performed in the SPEED mode for mass scans and in the ANSWER mode for SIM.

4. Liquid Scintillation Spectrometry

All liquid scintillation counting was performed on a Beckman LS 7000 liquid scintillation system equipped with a Datamex 43 printer terminal. The formula for scintillation fluid mixture is described in Appendix II.

5. Apparatus for Studies on Inhibition of Reuptake of Neurotransmitter Amines

The apparatus for the study of inhibition of reuptake of tritiated neurotransmitters consisted of a glass filtration flask (trap) attached to a vacuum and a filtration system obtained from the Millipore Corporation. The Millipore filtration system consisted of a 20 cm diameter tank, a cover with 12 spaces for glass fiber filters, and a head with screw-on compression nut. All components are made of plastic.

6. Apparatus for the Study of Release of Neurotransmitter Amines

The apparatus for the study of the release of neurotransmitters from nerve terminals was modeled after that of Raiteri *et al.* (1974). The basic set-up is described in Figure 5. In addition a Gilson Miniplus 2 peristalic pump and a LKB 7000 Ultrorac fraction collector were employed.

7. Balances

All qualitative and quantitative analyses were done by intially weighing standards to the nearest 0.1 mg on a Sartorius 2003 MPl scale.

8. Centrifugation

Centrifugation that was not done on a bench top centrifuge was carried out on a DAMON IEC B-20A centrifuge, with the head temperature kept at approximately 0°.

9. Miscellaneous Equipment

A McIllwain tissue chopper was used to make neuronal prisms from hypothalamic and striatal tissue for release and inhibition of reuptake experiments.

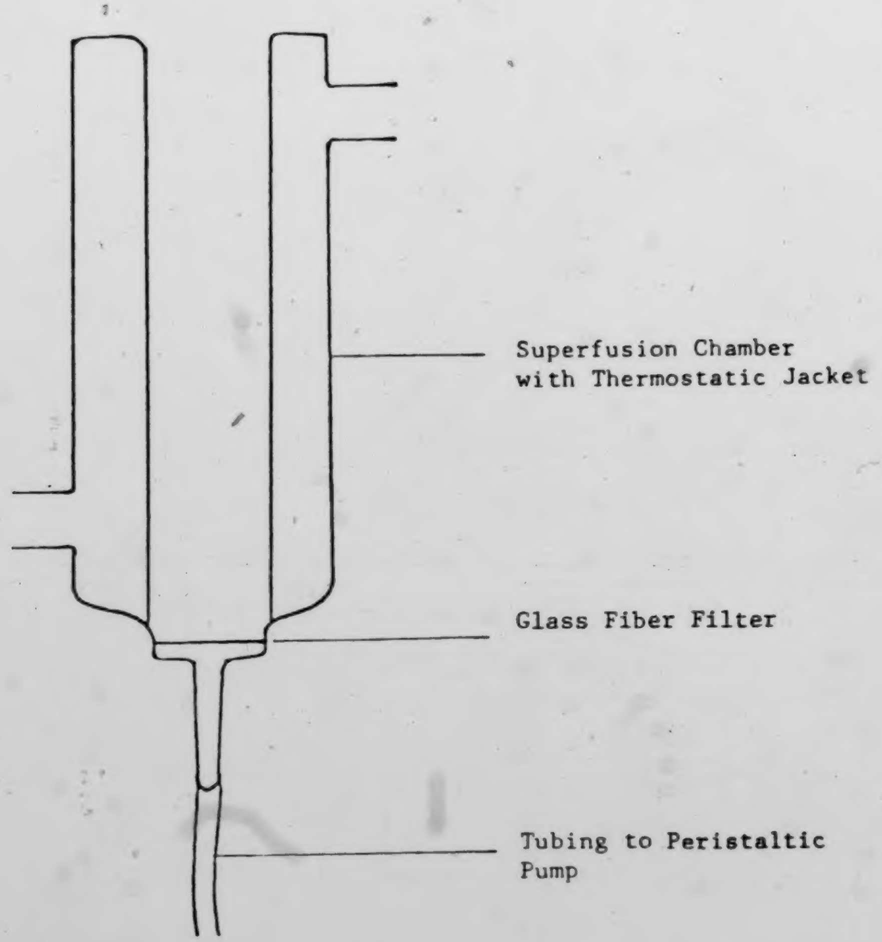

Superfusion Chamber
with Thermostatic Jacket
Glass Fiber Filter
Tubing to Peristaltic
Pump

Figure 5. An individual chamber of the apparatus used to study the release of tritiated neurotransmitters is shown above. The system is modelled after that used by Raiteri et al. (1974). Six of these individual glass chambers are arranged side by side. The thermostatic jacket is perfused with water maintained at 37°. The bottom section, onto which the glass fiber filter is placed, is removable. Tubing from the bottom of the filter holders is connected to a peristaltic pump which dispenses eluate into vials on a fraction collector.

II.E. Drug Synthesis

1. 6-Hydroxytetrahydro-β-Carboline

The synthesis of 6-OH-THBC was performed by the author using the method of Ho and Walker (1971). Briefly, 2.5 mmoles of 5-HT were dissolved in 8 ml of water at 45°. After cooling, 2.8 mmoles of glyoxylic acid in 0.6 ml of water were added, followed by the slow addition of 2.5 mmoles of KOH in 0.7 ml of water. Precipitation of tetrahydro-β-carboline-1-carboxylic acid took place during the addition of the KOH solution. After stirring at ambient temperature for 1 hr, the solid was collected on a filter and washed thoroughly with 2 ml of water. The damp filter cake was transferred to a beaker and suspended in 4.8 ml of water, and 0.7 ml of concentrated HCl was slowly added with stirring. The mixture was boiled on a hot plate for 30 min. and then an additional 0.7 ml of concentrated acid was added. Heating was continued for another 15 min. and the resulting solution was cooled to room temperature.

The preciptated HCl salt was collected and recrystallized in ethanol. The structure was confirmed by nuclear magnetic resonance (nmr) spectrometry and mass spectrometry. The nmr spectrum and melting point were in agreement with literature values (m.p. 272-275°) (Ho and Walker, 1971).

2. Para-Hydroxytranylcypromine

Synthesis of p-OH-TCP by Drs. Tse W. Hall and Ronald Micetich (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta) was performed by the following method: p-methoxycinnamic acid was converted into the corresponding acid chloride by reacting with thionyl chloride in refluxing benezene. The acid chloride was converted to the tert-butyl ester by reacting with an excess of dry tert-butanol in the presence of pyridine as base and catalytic amounts of 4-dimethylaminopyridine. Cyclopropanation of the *tert*-butyl *p*-methoxycinnamate was performed by using trimethylsulfonium iodide and sodium hydride in dimethyl sulfoxide (Kaiser *et al.*, 1965). The *tert*-butyl ester moiety was cleaved with trifluoroacetic acid to provide the cyclopropyl acid. The acid was again converted to the acid chloride and stirred with aqueous sodium azide in the presence of tetrabutylammonium chloride. In this way the acid chloride was smoothly transformed into the corresponding acyl azide. The acyl azide was then converted into the *tert*-butyl carbamate by heating with *tert*-butanol in benzene. The cleavage of the methoxy and *tert*-butyl carbamate groups was achieved by reacting with boron tribromide in methylene chloride at room temperature. After quenching with water, the aqueous layer was basified and the *p*-OH-TCP was extracted into $CHCl_3$ containing 20% di(2-ethylhexyl)phosphoric acid (DEHPA). Subsequently, it was converted to the hydrochloride salt by extracting with diluted HCl. Verification of the structure was confirmed by nmr, EI-MS, and CI-MS.

II.F. Drug and Drug Metabolites Analyses

1. Tranylcypromine, Tranylcypromine Isomers, and Amphetamine

The procedure used for the analysis of TCP, TCP isomers, and AMP in rat brain was that of Hampson *et al.* (1984a). Brains were weighed and homogenized in 5 volumes of ice-cold 0.1 N $HClO_4$ containing 10 mg % EDTA. Homogenates were transferred to cold centrifuge tubes and centrifuged at 10,000 rpm (12,000 x g) for 15 min. Four ml of the supernatant was taken for analysis. The supernatants were kept on ice and 500 ng of the internal standard *para*-chlorophenylethylamine (*p*-Cl-PEA) were added to each. The supernatants were neutralized with solid $KHCO_3$ and shaken with 5 ml of DEHPA (2.5% v/v in $CHCl_3$), and the mixture was centrifuged on a bench-top centrifuge for 5 min. The retained bottom layers were shaken with 0.5 N HCl (3 ml) for 4 min. After centrifugation, the top aqueous layers were basified with solid $NaHCO_3$ prior to the addition of 0.3 ml of acetic anhydride (AA). After acetylation was complete (cessation of CO_2 formation) the solutions were extracted with 4 ml of ethyl acetate. The organic layers were taken to dryness under a stream of N_2 and the residues reacted with pentafluorobenzoyl chloride (PFBZ) (2 µl in 300 µl of toluene) for 60 min. at 80°. After cooling, the mixtures were taken to dryness under a stream of N_2 and 300 µl of toluene added. An aliquot of this was taken for GC analysis. If samples appeared "dirty," additional clean-up could be accomplished by washing the final toluene extract with an equal volume of 1 N NH_4OH (in microfuge tubes).

2. (a) Para-Hydroxytranylcypromine in Urine

Para-hydroxytranylcypromine in urine was analyzed by the following method: urines were centrifuged on a bench-top centrifuge for 5 min. Aliquots of the supernatants (2.5 ml) were transferred to test tubes containing 2 µg of the internal standard nomifensine, and solid $KHCO_3$ was added until the pH was 7.8-8.0. After separation from the precipitate, the supernatants were shaken with 3 ml of a solution of DEHPA (2.5% v/v in $CHCl_3$) for 5 min., the mixtures were centrifuged, and the top layers were aspirated off. The bottom layers were extracted with 2 ml of 0.5 N HCl. The HCl layers were basified with solid Na_2CO_3 and then extracted with solutions (3 ml) of ethyl acetate-acetonitrile (9:1) containing 2 µl/ml of PFBZ. After separation of the layers by centrifugation, the organic layers were taken to dryness under N_2 and the residues taken up in 300 µl of toluene. One µl was taken for analysis by GLC or GC-MS. If additional clean-up was required, the final toluene extracts were shaken with an equal volume of 1.0 N NH_4OH in microfuge tubes.

(b) Para-Hydroxytranylcypromine in Brain

A procedure for the analysis of *para*-tyramine (Baker *et al.*, 1982) was used to analyze *p*-OH-TCP in brain tissue. Briefly, brain tissue was homogenized in 5 volumes of ice-cold 0.1 N $HClO_4$ containing 10 mg % EDTA. The homogenates were centrifuged (12,000 x g) for 15 min. Four ml of the supernatant were taken for analysis. Benzylamine (500 ng) was added to each supernatant as internal standard. The pH was adjusted to 7.8 with excess solid $KHCO_3$ and 400 µl of sodium phosphate buffer (0.25 M; pH 7.8). This mixture was shaken with 5.0 ml of a solution of DEHPA (2.5% v/v in $CHCl_3$) and the top layers aspirated off. To the bottom layers, HCl (0.5 N, 2.5 ml) was added. The tubes were shaken and the top HCl layers were removed and neutralized with $NaHCO_3$. Acetic anhydride (300 µl) was added with additional $NaHCO_3$ and the acetylation allowed to proceed for 20 min. The acetylated amines were then extracted with ethyl acetate (3 ml). To the ethyl acetate phases, NH_4OH (10 N, 400 µl) was added and the mixtures were shaken for 40 min., after which HCl (6 N, 300 µl) was added and shaken for an additional 15 sec. The organic layers were removed and evaporated to dryness under N_2. Ethyl acetate (25 µl) and trifluoroacetic anhydride (TFAA) (75 µl) were added to the residues and the reaction was allowed to proceed at room temperature for 30 min. Cyclohexane (300 µl) and saturated aqueous sodium tetraborate (3.0 ml) were added and the mixtures were shaken for 15 sec. The organic layers were removed and 1 μl was taken for GC or GC-MS analysis.

3. Analysis of 6-Hydroxytetrahydro-β-Carboline

The analysis of 6-OH-THBC in rat urine involved a two-step extraction-derivatization process. Initially, the urine samples were centrifuged on a benchtop centrifuge for 5 min. Two ml of each urine supernatant were then incubated with 660 μl of 1.0 N sodium acetate (pH = 6), 330 μl of 2% EDTA, and 66 μl of glusalase for 24 hrs at 37°. After the incubation period, 500 ng of the internal standard 5-methyltryptamine (5-MT) were added to each urine sample. Solid Na_2CO_3 and 3 ml of a solution containing 2 μl/ml of PFBZ in ethyl acetate:acetonitrile (9:1) were added. After agitating the mixtures for 8 min., the organic layers were separated, washed with 1 ml of water, and evaporated under a stream of N_2. The residues were taken up in 300 μl of toluene, washed rapidly with 300 μl of a 1 N NH_4OH solution and taken to dryness. These residues were then reacted with 75 μl of pentafluoropropionic anhydride (PFPA) and 25 μl of ethyl acetate for 30 min. at 60°. Excess PFPA was removed under a stream of N_2, the residues were redissolved in 200 μl of toluene, and aliquots were taken for analysis by GLC or GLC-MS on a fused silica capillary column.

II.G. Analysis of Biogenic Amines

1. Determination of Amines in Brain Tissue

(a) 5-Hydroxytryptamine and Tryptamine

A modification of the procedure of Baker *et al.* (1980) was employed. Frozen brains were thawed and homogenized in 5 volumes of ice-cold 0.1 N $HClO_4$ containing 10 mg % EDTA. The homogenates were centrifuged at 12,000 x g at 0° for 15 min. Five hundred ng of the internal standard 5-methoxytryptamine (5-MT) were added to the supernatants (3 ml), which were then neutralized with solid $KHCO_3$ and centrifuged to remove the $KClO_4$ precipitate. Sodium phosphate buffer (0.25 N, pH 7.8, 400 µl) and the liquid ion-pairing reagent DEHPA (2.5% v/v in $CHCl_3$, 4 ml) were added. The mixtures were shaken for 5 min., and the layers were separated in a bench-top centrifuge. The top layers were aspirated off and the bottom layers transferred to clean test tubes containing 2.5 ml of 0.5 N HCl. These mixtures were then shaken for 5 min. and the layers separated by centrifugation. The HCl layers were transferred to new tubes and neutralized with solid $NaHCO_3$. Acetylation was carried out by adding 300 µl of AA and additional $NaHCO_3$ until the reaction was complete (effervescence ceases). The aqueous layers were transferred to clean test tubes and 4 ml of ethyl acetate were added; the mixtures were shaken for 5 min. and the layers separated by centrifugation. The ethyl acetate layers were washed with 500 µl of water (to remove traces of $NaHCO_3$ and CH_3COONa) and then taken to dryness under a stream of N_2. The residues were reacted with 75 µl of PFPA and 25 µl of ethyl acetate at 60° for 30 min. These mixtures were cooled at room temperature and 300 µl of cyclohexane were added with shaking. Saturated sodium borate solution (3 ml) was added, and the mixtures were shaken for 30 sec. and centrifuged rapidly. The cyclohexane layers were retained and frozen until analysis on GC or GC-MS. On-column sensitivity was less than 10 pg.

(b) Dopamine

Frozen rat whole brains were thawed and homogenized in 5 volumes of ice-cold 0.1 N $HClO_4$ containing 10 mg % EDTA. The homogenates were centrifuged at 12,000 x g for 15 min. and 3.5 ml of the supernatant were transferred to 13 ml screw-top test tubes. A solution of sodium metabisulfite (10 mg/ml, 35 µl) and 500 ng of the internal standard N-methyldopamine were added to the tubes. Solid $KHCO_3$ was added until the pH was 8.0-8.2. The tubes were centrifuged on a bench-top centrifuge and the supernatants transferred to another set of tubes containing 100 mg of acidic aluminum oxide. The tubes were shaken for 5 min. and centrifuged again. The supernatants were aspirated off and 300 µl of 1 N HCl was added to each tube and the tubes were shaken for an additional 5 min. The tubes were centrifuged again and the HCl phases were transferred to new test tubes and taken to dryness under N_2. The residues were reacted with 75 µl of PFPA and 25 µl of ethyl acetate at 60° for 30 min. After cooling, the mixtures were taken to dryness under N_2. Toluene (200 µl) was added and the mixtures were vortexed for 1 min. and quickly centrifuged. A 1 µl portion of the top organic phase was taken for GC analysis. Test tubes were kept on ice at all times, until derivatization with PFPA. On-column sensitivity was less than 50 pg.

(c) β-Phenylethylamine

Phenylethylamine in brain tissue was analyzed using the method of Hampson *et al.* (1984b). Brains were homogenized in 5 volumes of ice-cold 0.1 N $HClO_4$ and centrifuged at 12,000 x g for 15 min. Three hundred ng of the internal standard *p*-Cl-PEA were added to the supernatants (4 ml), which were then neutralized with solid $KHCO_3$ and the precipitates discarded. The supernatants were shaken with DEHPA (2.5% v/v in $CHCl_3$, 4 ml) for 5 min. and centrifuged. The top aqueous layers were aspirated off, 3 ml of 0.5 N HCl were added, and the tubes were shaken for 4 min. After centrifugation on a bench-top centrifuge, the top layers were removed and neutralized with solid $NaHCO_3$. Acetic anhydride (0.3 ml) was added along with continuous addition of $NaHCO_3$ until the reaction was complete (Martin and Baker, 1977). Acetylated PEA was extracted with 4 ml of ethyl acetate, and the organic layers were washed with 0.5 ml of water and taken to dryness under N_2. The residues were reacted with PFBZ (2 µl in 300 µl of toluene) for 60 min. at 80°. After cooling, the mixtures were taken to dryness under N_2 and the residues redissolved in 300 µl of cyclohexane. One µl of this final extract was taken for GC or GC-MS analysis. Standard curves were constructed for each run. On-column sensitivity was less than 5 pg.

2. Amine Determinations in Urine

(a) Analysis of 5-Hydroxytryptamine and Tryptamine in Urine

The pH of the urine samples (4 ml) was adjusted to 7.8 by the addition of solid $KHCO_3$. The precipitates were separated by centrifugation and discarded. After the addition of 500 ng of the internal standard 5-MT, sodium phosphate buffer (0.25 M, pH 7.8, 400 µl), and DEHPA (2.5% v/v in $CHCl_3$, 5 ml) to the supernatants, the mixtures were shaken for 4 min. The layers were separated on a bench-top centrifuge and the top aqueous layers aspirated off. The bottom layers were transferred to another set of tubes containing 2.5 ml of 0.5 N HCl and mixed for 5 min. The layers were separated and the HCl layers removed and neutralized with solid $NaHCO_3$. After acetylation with AA (300 µl), the solutions were extracted with 4 ml of ethyl acetate. The organic layers were removed and washed with 800 μ l of water. The ethyl acetate layers were transferred to another set of tubes and taken to dryness under N_2. The resulting residues were reacted with 75 μ l of PFPA and 25 μl of ethyl acetate for 30 min. at 60°. After cooling, the derivatization mixtures were taken to dryness under N_2 and the residues redissolved in 300 μl of cyclohexane. The cyclohexane was quickly washed with 3 ml of a saturated sodium borate solution. An aliquot (1 μl) was taken for GC analysis.

(b) Analysis of 3-Methoxytyramine and Phenylethylamine in Urine

The procedures described by Coutts et al. (1981) and Baker et al. (1981) were used. The pH values of the urine samples (4 ml) were adjusted to 7.8 by adding solid $KHCO_3$. The precipitates were separated by centrifugation and discarded. Sodium phosphate buffer (0.25 M, pH 7.8, 400 μl), 1000 ng of the internal standard benzylamine, and DEHPA (2.5% v/v in $CHCl_3$, 5 ml) were added to the supernatants and shaken for 5 min. After centrifugation, the top aqueous layers were aspirated off and 2.5 ml of 0.5 N HCl were added to the organic layer. The mixtures were shaken for 4 min. and centrifuged. The top layers were transferred to other tubes and neutralized with solid $NaHCO_3$. Acetylation was performed by the addition of AA (300 μl) and the continuous addition of $NaHCO_3$ until the reaction was complete. The acetylated amines were shaken with 4 ml of ethyl acetate and the organic layers were transferred to another set of tubes. Hydrolysis of acetylated phenol groups was carried out by the addition of 400 μl of 10 N NH_4OH (agitation for 40 min.) (Coutts et al., 1980). The NH_4OH was neutralized with 660 μl of 6 N HCl. The ethyl acetate layers were removed to another set of tubes and taken to dryness under N_2. The residues were reacted with 75 µl of TFAA and 25 µl of ethyl acetate for 30 min. at room temperature. After the addition of 300 µl of cyclohexane, the mixture was vortexed; saturated sodium borate (3 ml) was added and the tubes quickly shaken and centrifuged. The layers were separated and an aliquot (1 µl) was taken for GC analysis. On-column sensitivity was less than 10 pg.

II.H. MAO Assays

MAO assays were performed on two types of tissue. In some experiments, brain tissue from rats pretreated with TCP (or its isomers) was used. In other experiments (termed *in vitro* experiments here) known amounts of drugs were added to tissue homogenates prepared from the brains of control, untreated rats.

Rat brains were homogenized in 25 volumes of ice-cold isotonic KCl and kept on ice. Solutions of a mixture of labelled and unlabelled substrates were prepared such that the final concentration of each substrate was 35 µM in the incubation tubes. Sodium phosphate buffer (0.5 M, pH 7.4, 250 µl) was added to the appropriate tubes and placed on ice. For *in vitro* assays, the test drugs were added at this time. Isotonic KCl (25 µl) was added to blanks and 25 µl of the brain tissue homogenate were added to all other tubes. (For *in vitro* assays, the drugs were incubated with the tissue in the tubes at this stage for 10 min. at 37°).

Aliquots (25 µl) of either the C^{14}-5-HT or C^{14}-PEA substrate mix were added to all tubes; the tubes were then incubated in a water bath for 20 min. at 37°. The tubes were cooled to room temperature and 200 µl of 2 N HCl were added to each to stop the reaction. Toluene (6 ml) was added, the mixture was vortexed, and the tubes were placed in the freezer at -50° until the aqueous layer was frozen. The toluene was decanted into scintillation vials containing 9 ml of scintillation fluid, and the radioactivity was counted in a liquid scintillation counter.

II.I. Determination of the Release of Tritiated Neurotransmitter Amines from Brain Tissue

Rats were sacrificed by cervical fracture, and the brains were removed and placed on an ice-cold plate. Either the corpus striatum (for determination of release of DA or 5-HT) or the hypothalamus (for determination of release of NE) was then dissected out. The tissues were chopped into prisms (0.1 x 0.1 x 2 mm) on a McIlwain tissue chopper and dispersed in ice-cold incubation medium of the following composition: 123 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM glucose, 1 mM ascorbic acid, 12.5 μM nialamide, and 20 mM Tris-HCl buffer, pH 7.4. The tissues were further diluted with medium to give a concentration in the flasks of 5 mg/5 ml. The flasks were pre-incubated for 10 min. at 37°, at which time the radiolabelled neurotransmitter was added (final concentration: 0.02 μM).

The samples were incubated again for 7 min. and then poured onto Millipore filters in thermostatically jacketed superfusion chambers (Figure 5). After washing the tissues on the filters with medium (2 x 5 ml) at 37°, release was investigated using the superfusion method of Raiteri _et_ _al._ (1975). This consisted of attaching the bottom stems of the chamber to a peristalic pump, superfusing the tissue with 0.5 ml/min. of incubation mix containing drug solutions or incubation mix alone (controls), and collecting the superfusates in successive 1 min fractions. Superfusion and collection of 1 min. fractions were carried out for a further 10 min. At the end of the superfusion period, the percentage of radioactivity in each fraction and that remaining on the filter was counted. The radioactivity in each fraction was then expressed as a percentage of the total radioactivity (fractions plus filter). The per cent in the first five fractions was summed in the controls and in the sample fractions, and the samples were expressed as per cent of control.

II.J. Determination of the Inhibition of Reuptake of Tritiated Neurotransmitters from Brain Tissue

The tissue homogenates were prepared as in section II.I to give a final concentration of 5 mg tissue/ml of incubation mix. The homogenates were made from tissue dissected from corpus striatum for DA and 5-HT experiments and from hypothalamus for NE experiments.

The tissue homogenates (1 ml) were added to 25 ml flasks containing 4 ml of incubation mix. Flasks containing 5 ml of incubation mix were used as blanks. The flasks were incubated in a water bath for 10 min. at 37°. The drug solutions were then added to the appropriate flasks, followed by the addition of the radiolabelled substrate mixture (final concentration 0.02 μM). The flasks were incubated for 7 min. at 37°, after which the contents of each were placed into the receptacle well of the Millipore filtration apparatus with the vacuum on. Each flask was rinsed twice with 5 ml of warm (37°) incubation mix and the rinses poured through the filtration apparatus. The glass fiber filters were removed and placed into 10 ml of scintillation fluid and counted in a liquid scintillation counter.

The blanks were subtracted from all samples, and then the drug-treated samples were expressed as a per cent of control averages. These values were subtracted from 100 to express data as per cent uptake inhibition.

II.K. Statistical Analysis

All statistical analysis was performed on a Texas Instrument TI Programable 58C calculator. Procedures for statistical analysis are presented in Appendix I. Error bars in all figures represent the standard error of the mean.

RESULTS

III. RESULTS

III.A. Acute and Chronic Treatment with Tranylcypromine

1. Animal Weights

Certain groups of animals in the chronic studies were weighed before, during, and after a 21-day dosing period. The results are presented in Table V. Statistical analysis (t-test, $p < 0.05$; see Appendix I) revealed that the control (saline-treated) group did not differ from the TCP-treated group at the beginning of the dosing regimen nor at the end of the dosing regimen (21 days). However, the difference scores, that is, the weight each rat gained over the 21-day dosing period, did differ. The TCP-treated group gained significantly less than the control group ($p < 0.01$). This is consistent with the anorexic effect of TCP and other MAOIs reported by others (Willis and Smith, 1982).

2. Levels of Tranylcypromine in the Brain

An extremely sensitive and reproducible method for the analysis of TCP in brain tissue was successfully developed (Hampson _et al._, 1984a). This method could also be adapted for the analysis of PEA (Hampson _et al._, 1984b) and AMP. Initial attempts to develop a TCP assay using a phase-transfer reaction with PFBZ were unsuccessful due to an endogenous interfering peak in the chromatogram. Aqueous acetylation followed by extraction and derivatization with PFBZ eliminated this problem.

The limits of detection of this method were approximately 0.75 ng/g brain tissue for both TCP and PEA. The advantages of this method over a previously developed method (Calverley _et al._, 1981) were lower limits of detection and applicability to the analysis of AMP. In addition, the

Table V. Comparison of rat weights and weight gains in rats treated chronically with saline or tranylcypromine for 21 days. Weights and weight gains are in grams. See Results, section III.A.1 for further details.

	Saline-Treated		Tranylcypromine-Treated	
	Rat	Weight	Rat	Weight
DAY 1	A	211	F	209
	B	220	G	230
	C	224	H	216
	D	216	I	217
	E	215	J	218
		x = 217.2		x = 218.0
DAY 21	A	308	F	301
	B	325	G	323
	C	328	H	301
	D	308	I	298
	E	318	J	308
		x = 317.4		x = 306.2
Weight Gain	A	97	F	92
	B	105	G	93
	C	104	H	83
	D	93	I	82
	E	102	J	91
		x = 100.2		x = 88.2[a]

[a] Significantly different from control $p < 0.01$.

derivatives were stable at 5° for at least two months. The acetylated, pentafluorobenzoylated derivatives of TCP, PEA, and AMP all gave characteristic diagnostic ions when subjected to EI mass spectrometry (Figures 6-8) and CI mass spectrometry (Figures 9-11). When p-Cl-PEA was used as internal standard, TCP and PEA could be assayed simultaneously. A gas chromatogram of a brain sample from a rat dosed with 1 mg/kg of TCP is shown in Figure 12.

The levels of TCP in rat whole brain tissue after treatment for 1, 10, 21, and 42 days are shown in Figure 13. Levels of TCP continued to increase throughout the 42-day dosing period, although after 21 days the increases were much less than those observed during the first 21 days, during which time the TCP level doubled from the initial dose. At each successive time interval, TCP levels differed significantly from the immediately previous time interval (t-test, $p < 0.05$), except between 21 and 42 days.

3. Inhibition of Monoamine Oxidase after Acute and Chronic Treatment with Tranylcypromine

During the time intervals mentioned in the above sections, the extent of inhibition of MAO in the brain was also measured. The results are presented in Table VI. A single injection of 1 mg/kg of TCP caused 86.8% and 86.9% inhibition of MAO-A and MAO-B respectively. There was no significant difference in the extent of inhibition of MAO-A versus MAO-B at any time interval throughout the study, that is, there was no selectivity. Monoamine oxidase inhibition continued to increase slightly throughout the 42-day period. By day 21, MAO-A was significantly more inhibited than after a single dose. For MAO-B, 42 days were

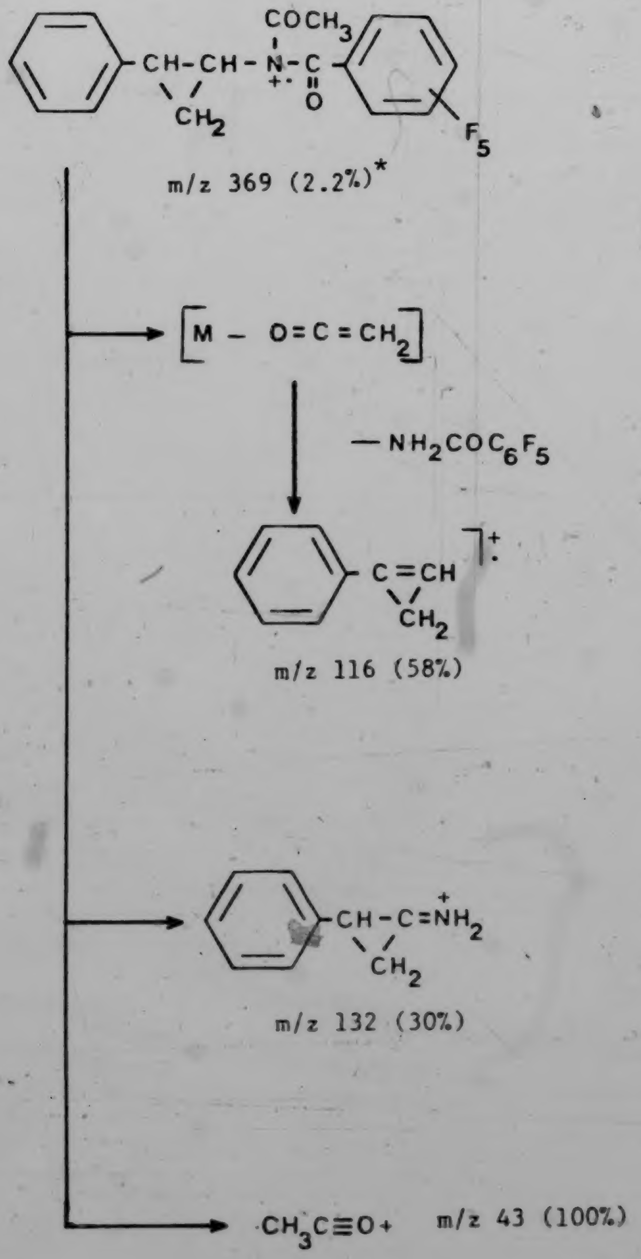

COCH3
CH-CH-N-C
+·
O
CH2
F5
m/z 369 (2.2%)*
[M - O=C=CH2]
— NH2COC6F5
C=CH
CH2
+·
m/z 116 (58%)
CH-C=NH2
+
CH2
m/z 132 (30%)
·CH3C≡O+
m/z 43 (100%)

Figure 6. Fragmentation pattern of the electron-impact mass spectrum of derivatized tranylcypromine. *Numbers in parenthesis indicate per cent relative abundance.

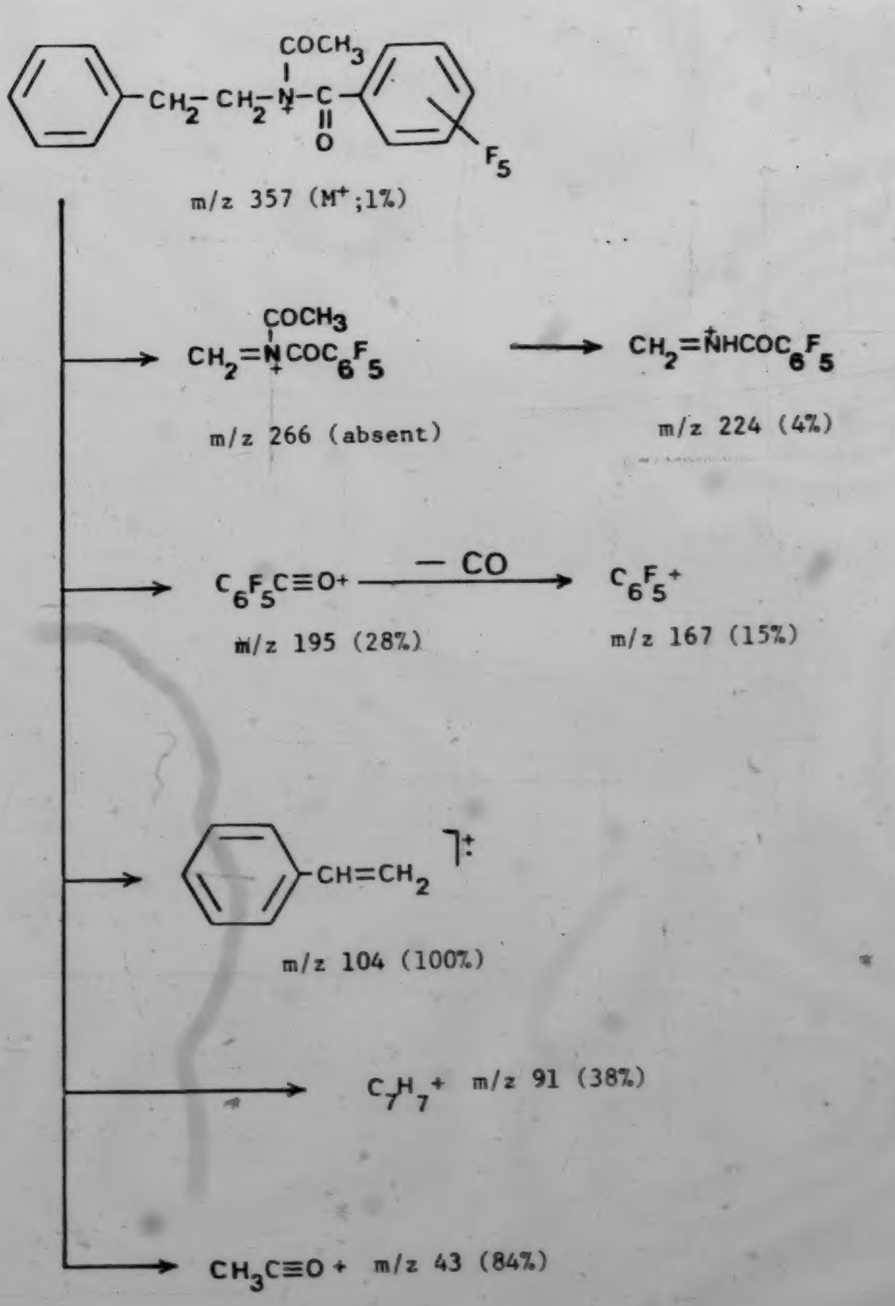

COCH3
CH2–CH2–N–C
O
F5
m/z 357 (M+;1%)
COCH3
CH2=NCOC6F5
m/z 266 (absent)
CH2=NHCOC6F5
m/z 224 (4%)
C6F5C≡O+
– CO
C6F5+
m/z 195 (28%)
m/z 167 (15%)
CH=CH2
m/z 104 (100%)
C7H7+ m/z 91 (38%)
CH3C≡O+ m/z 43 (84%)

Figure 7. Fragmentation pattern of the electron-impact mass spectrum of derivatized phenylethylamine.

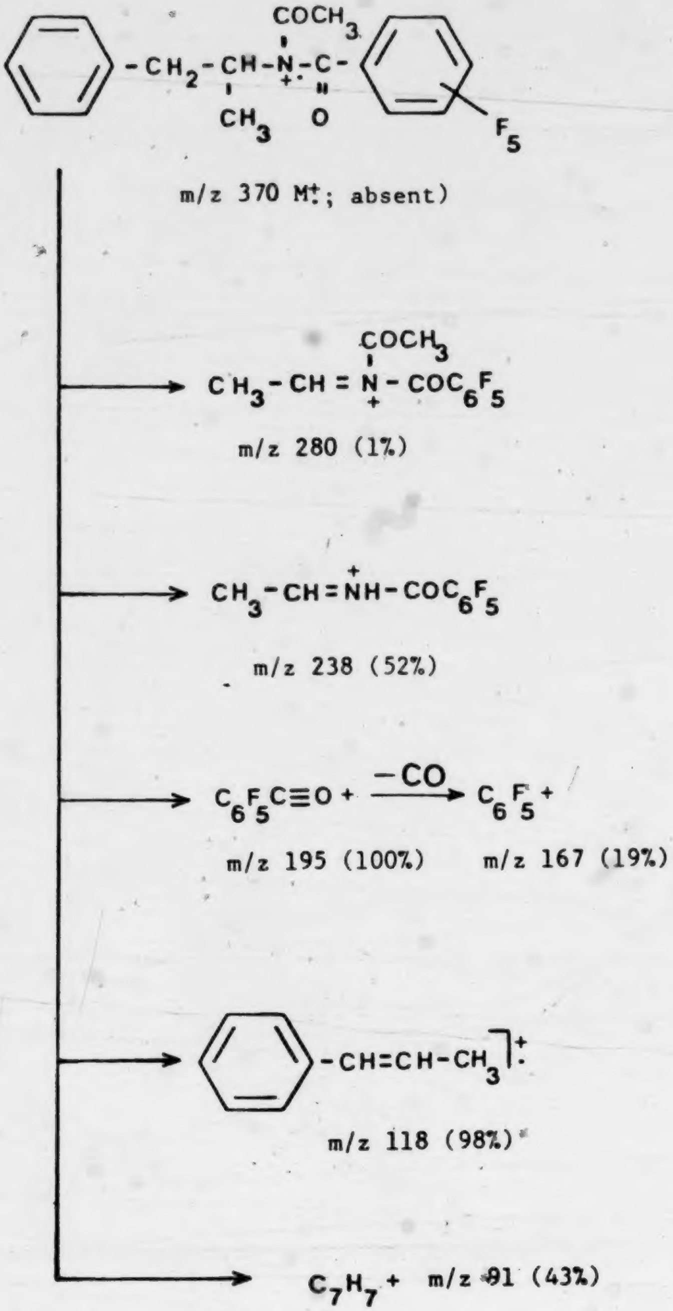

COCH3
CH2 CH N C
CH3 O
F5
m/z 370 M+; absent)
COCH3
CH3-CH = N - COC6F5
m/z 280 (1%)
CH3-CH=NH-COC6F5
m/z 238 (52%)
C6F5C≡O+
-CO
C6F5+
m/z 195 (100%)
m/z 167 (19%)
-CH=CH-CH3
m/z 118 (98%)
C7H7+ m/z 91 (43%)

Figure 8. Fragmentation pattern of the electron-impact mass spectrum of derivatized amphetamine.

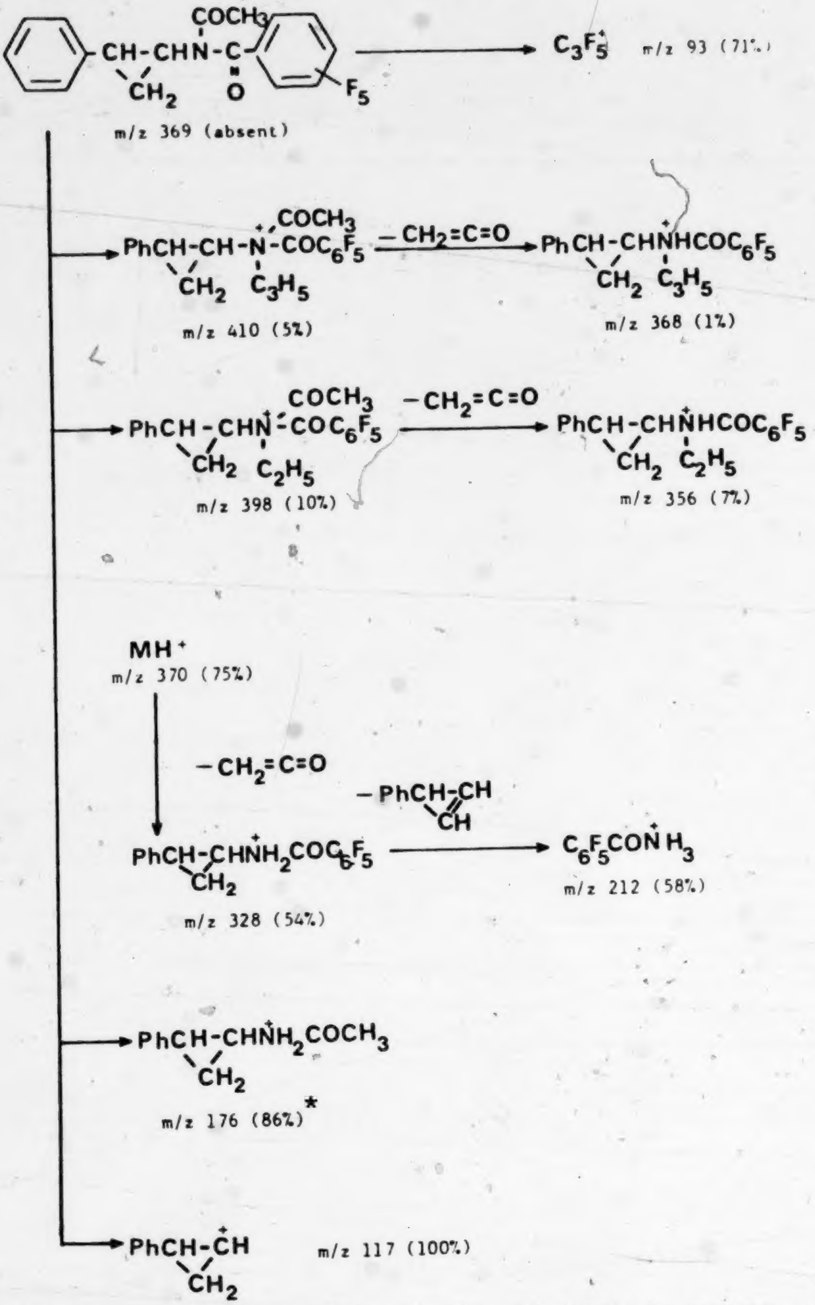

COCH3
CH-CHN-C-
CH2 O F5
m/z 369 (absent)
C3F5+ m/z 93 (71%)
PhCH-CH-N-COC6F5 COCH3 CH2 C3H5
m/z 410 (5%)
− CH2=C=O
PhCH-CHNHCOC6F5 CH2 C3H5
m/z 368 (1%)
PhCH-CHN-COC6F5 COCH3 CH2 C2H5
m/z 398 (10%)
− CH2=C=O
PhCH-CHNHCOC6F5 CH2 C2H5
m/z 356 (7%)
MH+
m/z 370 (75%)
− CH2=C=O
− PhCH-CH CH
PhCH-CHNH2COC6F5 CH2
m/z 328 (54%)
C6F5CONH3
m/z 212 (58%)
PhCH-CHNH2COCH3 CH2
m/z 176 (86%)*
PhCH-CH CH2
m/z 117 (100%)

Figure 9. Fragmentation pattern of the chemical ionization mass spectrum of derivatized tranylcypromine.*

*It would be unusual for this fragment to occur, and to ensure that it was not coming from contamination of the sample by N-acetyl-TCP, authentic N-acetyl-TCP was prepared and run under the same chromatographic conditions as N-acetyl,N-PFBZ-TCP. The two compounds separated completely, indicating that the frament indicated above is formed from N-acetyl,N-PFBZ-TCP in the mass spectrometer.

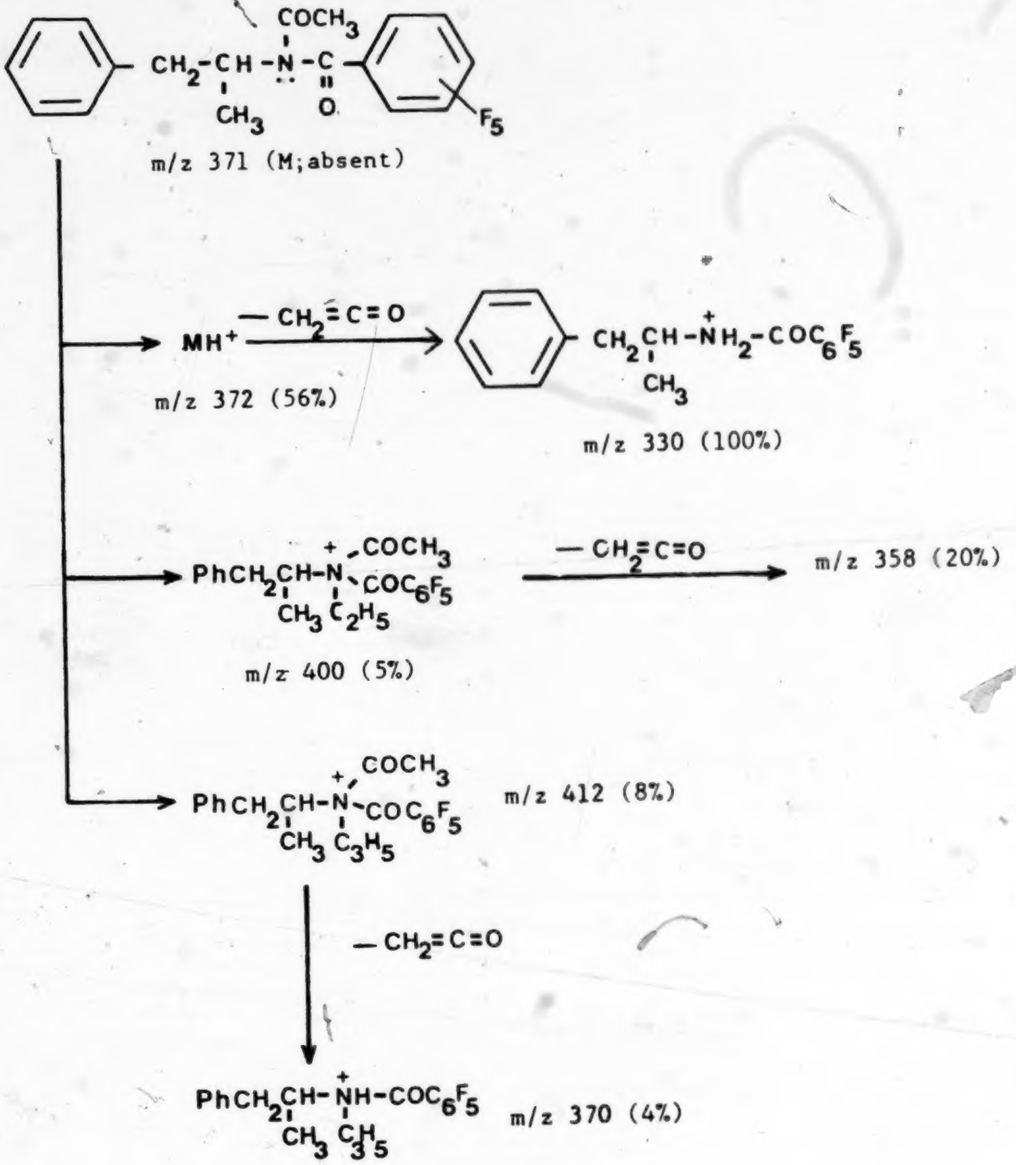

m/z 371 (M;absent)
MH+
— CH2=C=O
m/z 372 (56%)
m/z 330 (100%)
m/z 400 (5%)
— CH2=C=O
m/z 358 (20%)
m/z 412 (8%)
— CH2=C=O
m/z 370 (4%)

Figure 10. Fragmentation pattern of the chemical ionization mass spectrum of derivatized amphetamine.

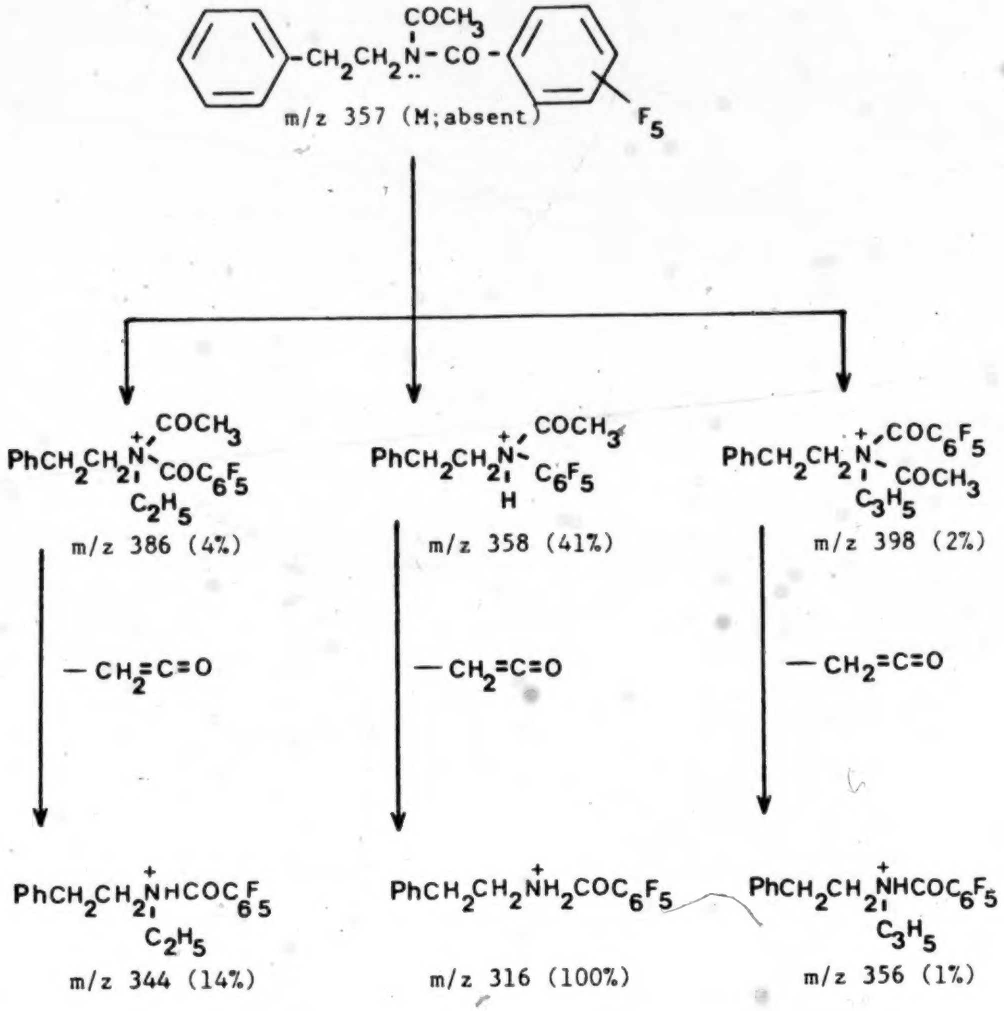

m/z 357 (M;absent)
m/z 386 (4%)
m/z 358 (41%)
m/z 398 (2%)
— CH₂=C=O
— CH₂=C=O
— CH₂=C=O
m/z 344 (14%)
m/z 316 (100%)
m/z 356 (1%)

Figure 11. Fragmentation pattern of the chemical ionization mass spectrum of derivatized phenylethylamine.

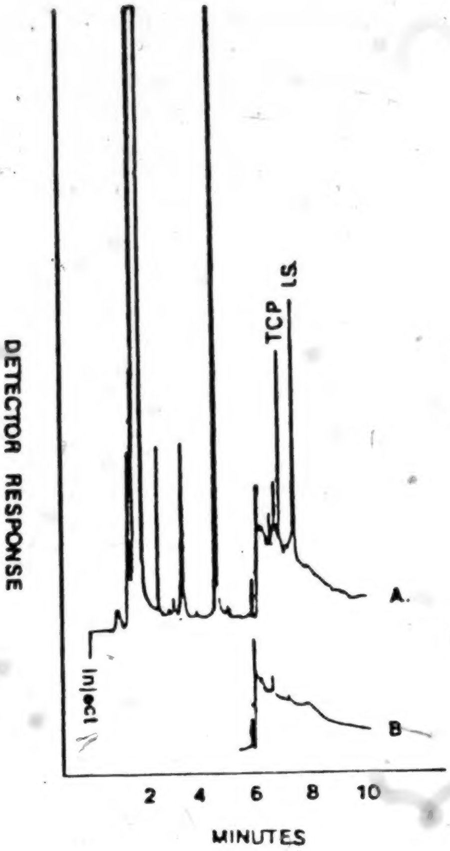


Figure 12. A gas chromatogram of a brain sample from (A) a rat dosed with 1 mg/kg of tranylcypromine and sacrificed 1.5 hours later, and (B) a control brain sample.

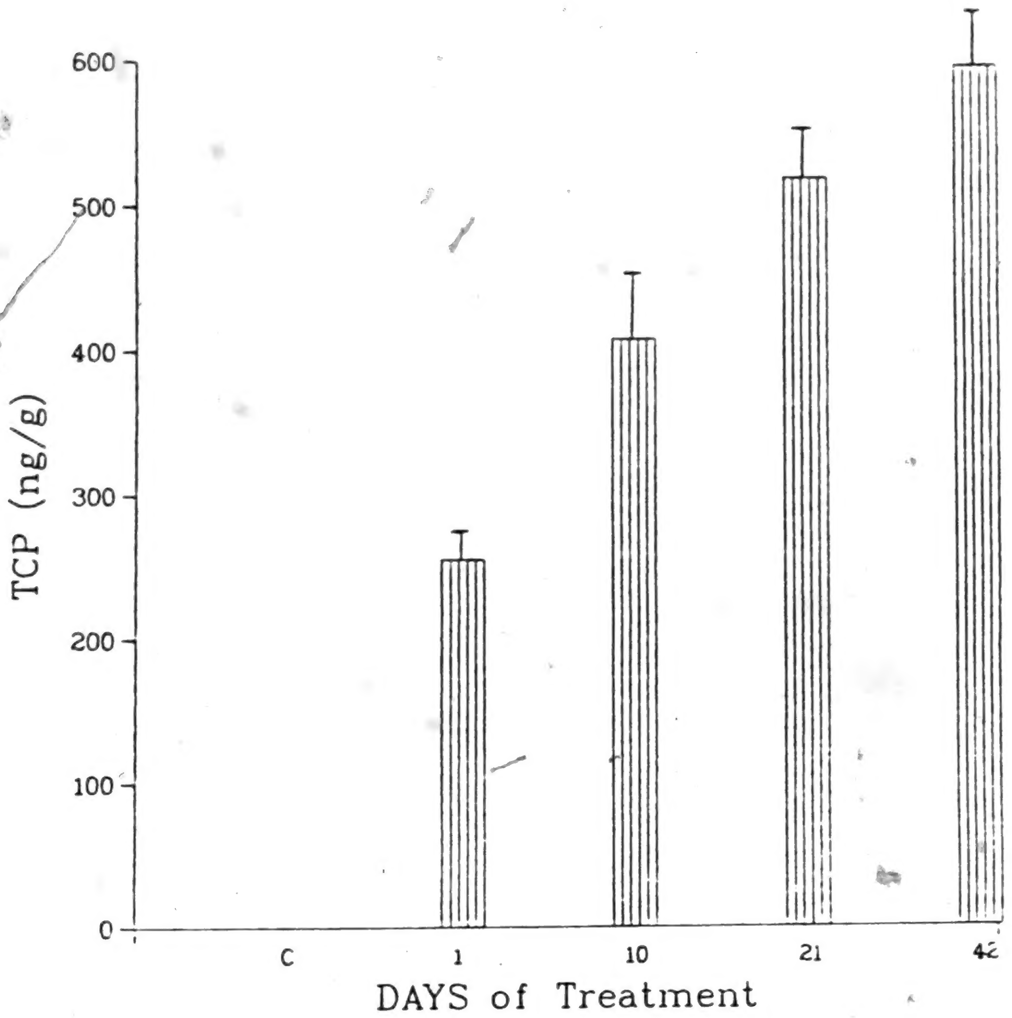


Figure 13. Levels of tranylcypromine in rat brain after acute and chronic treatment with tranylcypromine. N = 4-12.

Table VI. Inhibition of monoamine oxidase in vivo by acute and chronic treatment with tranylcypromine. Values represent the mean of the per cent inhibition (± S.E.M.). N = 5-16. ^{14}C-5-HT was used as MAO-A substrate and ^{14}C-PEA was used as MAO-B substrate.

	MAO-A	MAO-B
Day 1	86.8 (1.7)	86.9 (1.8)
Day 10	88.0 (1.8)	89.5 (1.4)
Day 21	91.3 (1.3)	89.8 (0.9)
Day 42	95.4 (1.1)	92.7 (1.1)

required to reach statistical significance ($p < 0.05$). The highest level of MAO inhibition (95.4% for MAO-A and 92.7% for MAO-B) was reached at the end of the 42-day dosing period. Only one sample in the entire study (MAO-A, 42-day) was 100% inhibited. The per cent inhibition (average MAO-A and MAO-B) correlated highly with the level of TCP in the brain ($r = 0.9519$, $p < 0.05$) over the 42-day dosing regimen.

4. Brain Levels of Biogenic Amines after Acute and Chronic Inhibition of Monoamine Oxidase

(a) β-Phenylethylamine and Tryptamine

The levels of PEA and T in rat whole brains after acute and chronic treatment with TCP are shown in Figure 14. The sensitivity of the analytical method for PEA allowed quantitation of this amine in whole brains of untreated rats. Tryptamine could not be detected in untreated rats, i.e., levels were < 1 ng/g. Levels of PEA increased 19-fold above control values after a single i.p. dose of 1 mg/kg, and these levels were maintained at approximately this value throughout the 42-day dosing period. There was no significant difference in PEA levels at any time interval between 1 and 42 days.

Tryptamine was detected in all drug-treated samples. Tryptamine levels increased up to day 21 and then decreased slightly by day 42; however, there was no significant difference between T levels between day 21 and day 42. In general, T values varied considerably from sample to sample, even within a time period. Although MAO-A and MAO-B were inhibited to approximately the same extent throughout the study, the MAO-B substrate PEA was substantially more responsive to MAO inhibition than was T, indicating that PEA may have a higher turnover rate than T.

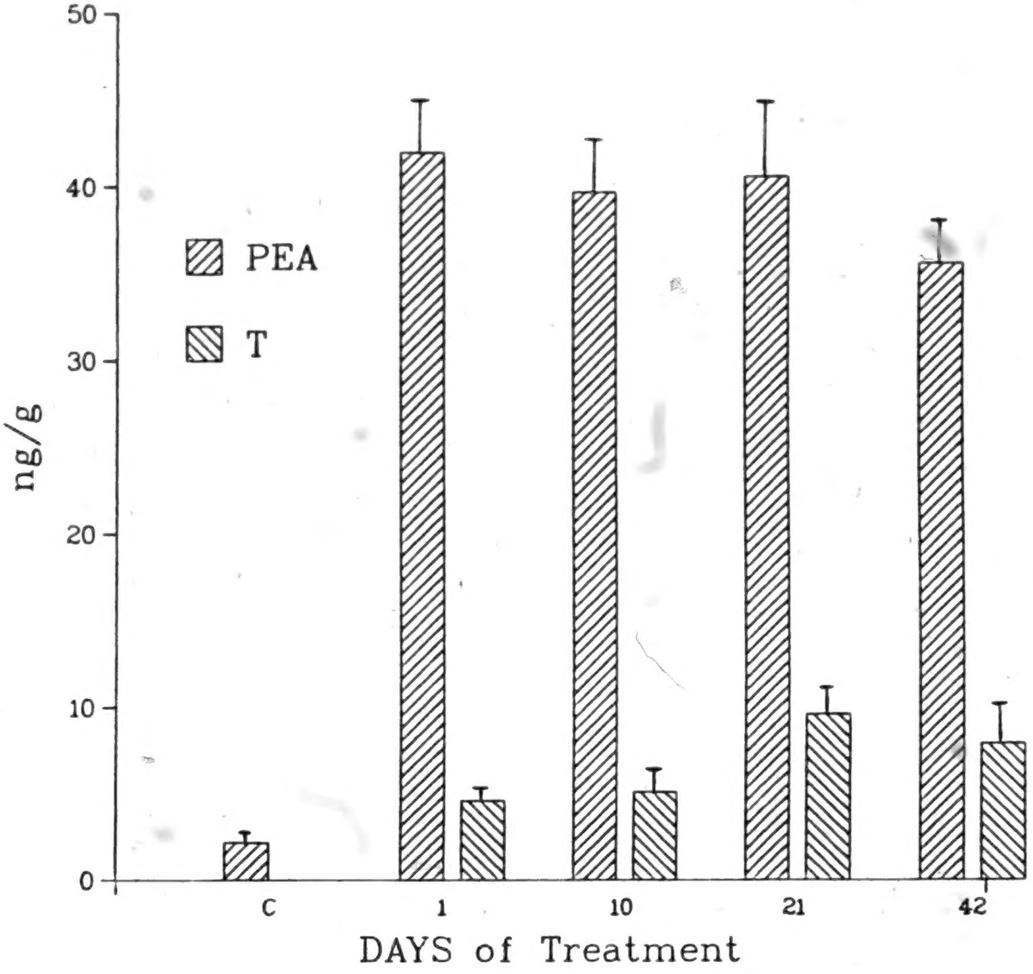


Figure 14. Levels of phenylethylamine and tryptamine in rat brain after acute and chronic treatment with tranylcypromine. Control levels of tryptamine were less than 1 ng/g. N = 4-7.

These results are in agreement with those of Durden and Philips (1980) who reported that PEA had a greater turnover rate (1.53 nmol/g/hr), 6x higher than that of T (0.24 nmol/g/hr).

(b) 5-Hydroxytryptamine

The levels of 5-HT in rat brains after acute and chronic treatment with TCP are shown in Figure 15. A single dose of TCP of 1 mg/kg increased brain 5-HT levels to 1.5x control values. Concentrations of 5-HT continued to increase throughout the study. However, although at each subsequent time interval the 5-HT level was significantly greater than at the previous interval, this effect disappeared between 21 and 42 days of dosing. In fact, the increases in the 5-HT levels in the brain had obviously plateaued by day 21. This pattern of 5-HT levels throughout the 42-day dosing period correlated highly with the brain TCP level ($r = 0.9640$, $p < 0.05$). No other biogenic amine measured in the chronic TCP study correlated as highly with brain drug levels over time.

(c) Dopamine

The levels of DA in rat brain after acute and chronic treatment with TCP are shown in Figure 16. A single dose of TCP increased brain DA concentrations to 1.4x control values. In all drug-treated animals, brain DA concentrations were significantly elevated above control levels. A maximum value (1207 ng/g) was obtained at 10 days, after which the DA concentration stayed at approximately this same level. Although the level dropped slightly at 21 days, after 42 days of dosing the brain DA was essentially equivalent to the level observed after 10 days of dosing. As was observed with the other amines measured, no trend

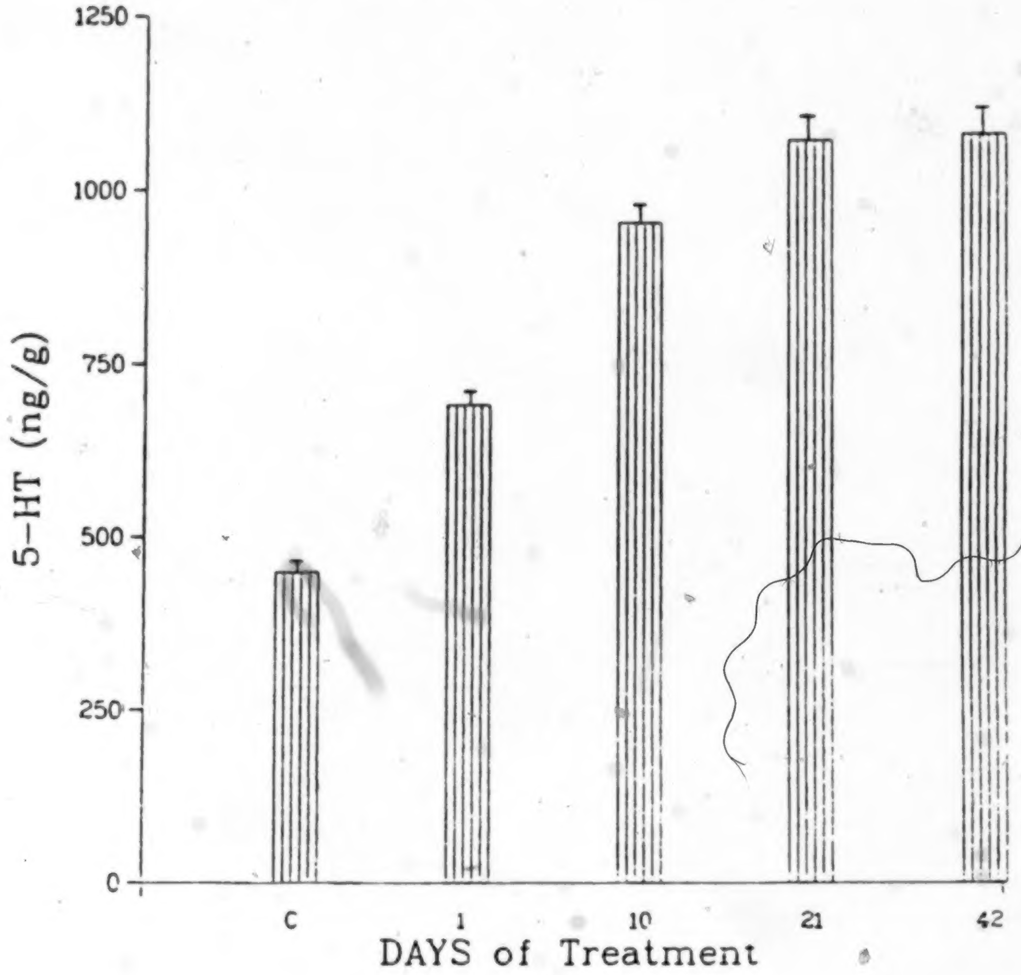


Figure 15. Levels of 5-hydroxytryptamine in rat brain after acute and chronic treatment with tranylcypromine. N = 6-12.

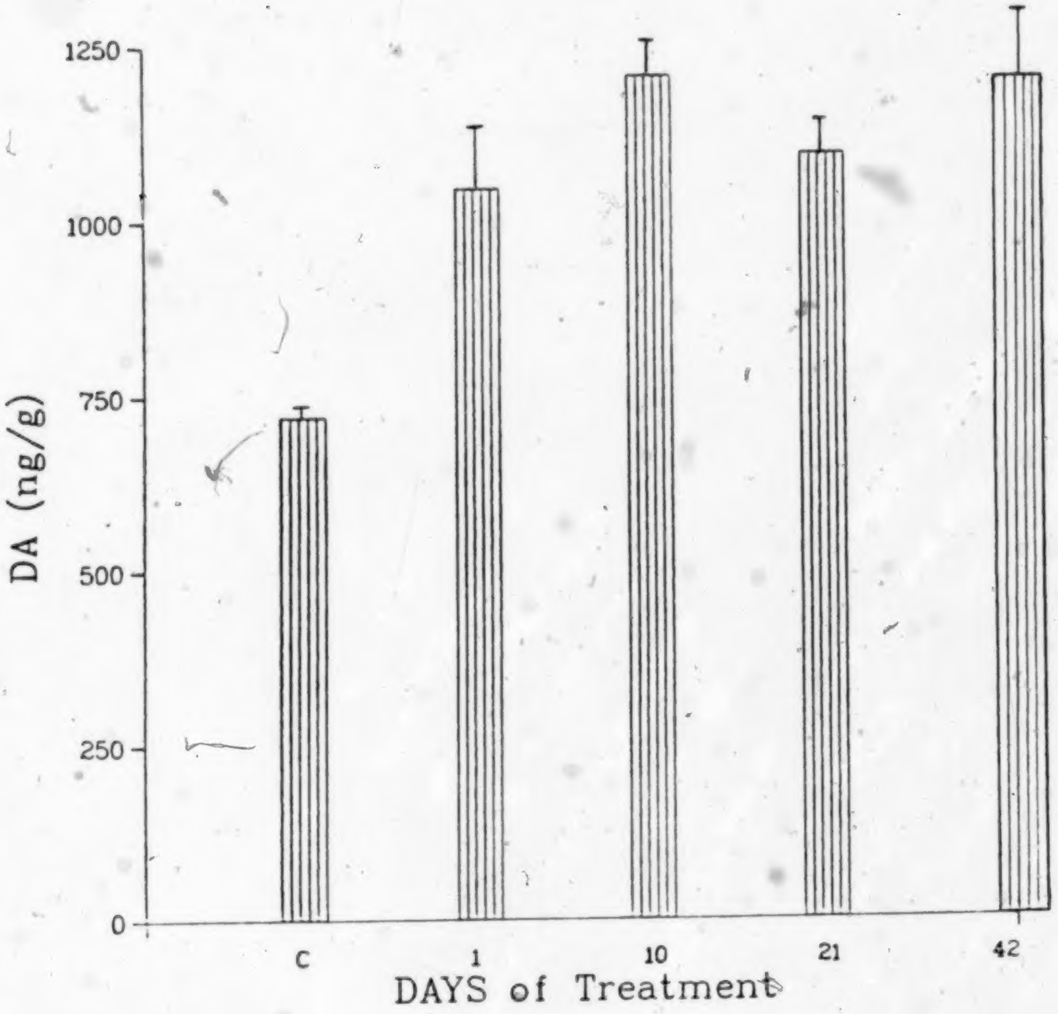


Figure 16. Levels of dopamine in rat brain after acute and chronic treatment with tranylcypromine. N = 5-9.

towards a return to baseline (control values) was observed.

5. Urinary Amine Levels after Acute and Chronic Treatment with Tranylcypromine

Levels of amines were also quantitated in 24-hr urine samples collected during chronic treatment with TCP. Baseline (control) values and levels at days 1, 10, and 21 were determined. All values are expressed as μg excreted per 24 hr.

(a) β-Phenylethylamine and Tryptamine

The amounts of PEA and T excreted in the urine during acute and chronic TCP treatment are presented in Figure 17. A single dose of TCP of 1 mg/kg caused urinary levels of PEA to increase to 3.8x control values. By day 10 this increase was 6.2x that of control values. On day 21, this increase in urinary PEA excretion had decreased slightly, indicating a plateau had been reached. Excretion of PEA on day 21 did not differ significantly from that on day 10. In contrast, PEA excretion on day 10 differed significantly from that on day 1 ($p < 0.025$). Levels of PEA at all time intervals from day 1 onwards were significantly greater than control values.

There are therefore differences in the effects of TCP on PEA levels between the brain and urine. While a fairly constant level of PEA was reached in the brain after a single dose of 1 mg/kg and maintained at approximately the same level for up to 42 days, the urinary levels increased significantly from day 1 to day 10. A plateau was apparently reached on or before day 10. There was no obvious trend towards a return to baseline levels in the brain after 42 days of dosing or in the

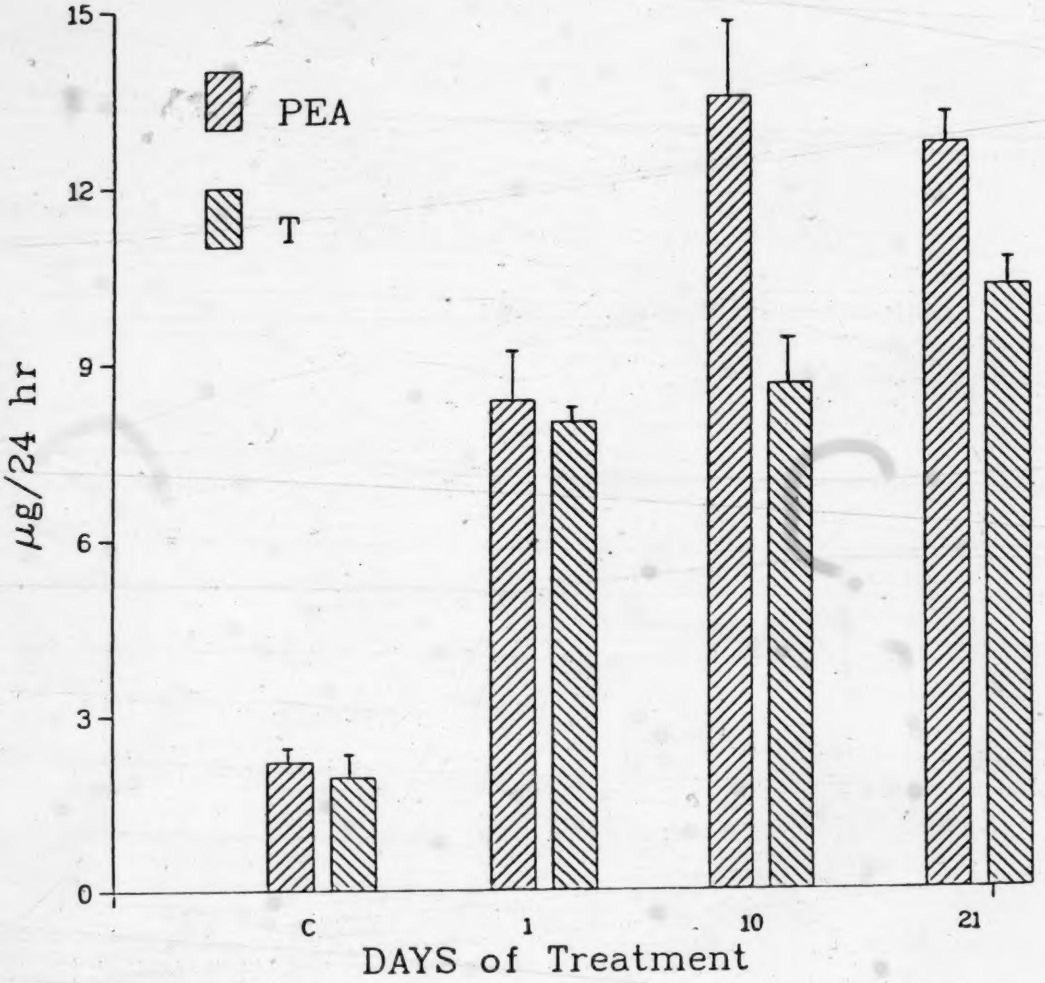


Figure 17. Urinary phenylethylamine and tryptamine after acute and chronic treatment with tranylcypromine. N = 4-6.

urine after 21 days of dosing.

Urinary T levels increased to 4.2x control values after a single dose of 1 mg/kg. At all time intervals, T levels were significantly elevated above control values. Concentrations of T at each successive time interval did not always differ from the previous interval, but the T level on day 1 did differ significantly from day 21.

(b) 5-Hydroxytryptamine and 3-Methoxytyramine

The values for 24-hr excretions of 5-HT and the DA metabolite 3-MT after acute and chronic TCP treatment are shown in Figure 18. In contrast to the large differences between 5-HT and T levels in the brain, urinary 5-HT and T levels in untreated rats were very similar. Their response to TCP was also similar. Although the initial increase in urinary 5-HT levels after a single dose of TCP was less dramatic than the T response (1.4x controls for 5-HT),it was significantly elevated above controls, as were 5-HT levels at all subsequent time periods. As with T, 5-HT levels between successive time intervals did not differ significantly from the time intervals immediately preceding; a plateau appeared to be reached within the first few days of dosing. This contrasted with the pattern of 5-HT levels in the brain in response to chronic TCP treatment, where a gradual and steady increase was seen until the plateauing at 21 days. The trend in urinary 3-MT levels appeared similar to that of brain DA concentrations in that the peak value was obtained after 10 days of dosing. Unfortunately, due to the wide variation observed in 3-MT excretion, no groups differed significantly ($p > 0.05$) from one another, including controls versus drug-treated animals.

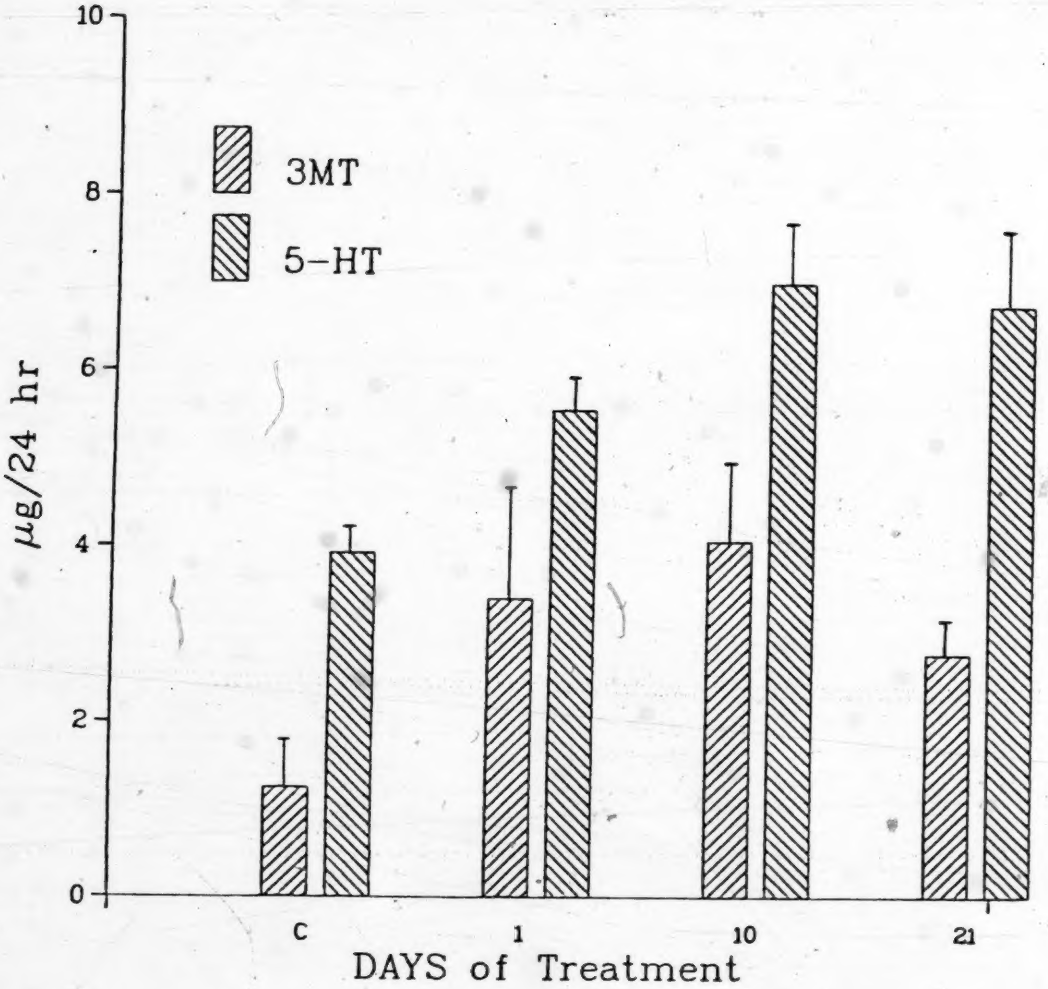


Figure 18. Urinary 5-hydroxytryptamine and 3-methoxytyramine after acute and chronic treatment with tranylcypromine. N = 3-5.

III.B. Administration of Tranylcypromine Isomers

The individual (+) or (-) isomers of TCP were administered (1 mg/kg) to groups of rats which were sacrificed 0.5, 1.5, 3.0, and 6.0 hr later. Various parameters were measured at these time intervals. The results are reported below.

1. Levels of Tranylcypromine Isomers in the Brain

The levels of the (+) or (-) isomers of TCP in the brain after administration of the respective isomer are displayed in Figure 19. Surprisingly, there were large differences in the disposition of the isomers. (-)-Tranylcypromine appeared to enter the brain faster, attain a higher level, and be cleared faster than the (+) isomer. By plotting the log of the isomer concentration against time, the half-life of (-)-TCP was determined to be 0.76 hr (45.7 min). By 6 hr after drug injection, the level of (-)-TCP in the brain was 5 ng/g, a level that is approaching the limits of detection of the assay. Considering that at 0.5 hr after injection, the level of (-)-TCP was approaching 1 μg/g, within 6 hours the drug level had decreased by a factor of about 200.

In contrast, the (+)-isomer attained a lower peak level and was cleared from the brain more slowly. The half-life of (+)-TCP in the brain was determined to be 1.45 hr (87 min), a value almost double that of the (-)-isomer. In addition, at the 6 hr time interval, the level of (+)-TCP was almost 10x greater than that of (-)-TCP. At the 0.5 hr time interval, (-)-TCP levels were significantly greater ($p < 0.02$) than those of (+)-TCP; at all other time intervals, (+)-TCP levels were significantly greater than (-)-TCP levels (1.5 hr, $p < 0.001$; 3.0 hr, $p < 0.001$; 6.0 hr, $p < 0.025$).

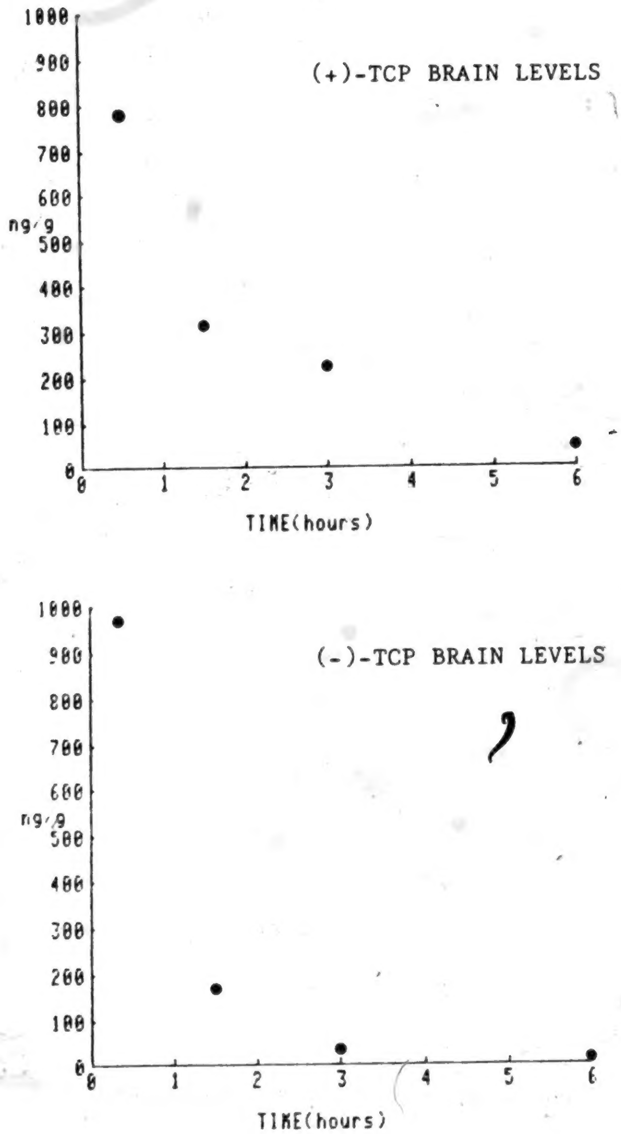


Figure 19. Levels of tranylcypromine iso[illegible] rat brain after dosing with tranylcypromine isomers.N[illegible]

2. Inhibition of Monoamine Oxidase by Tranylcypromine Isomers

Inhibition of monoamine oxidase by TCP isomers was determined both *in vivo* and *in vitro*. For *in vivo* experiments rats were treated intraperitoneally with 1 mg/kg of either (+)- or (-)-TCP and sacrificed at various time intervals after drug injection. The results of the *in vivo* MAO inhibition experiments are presented in Table VII. The (+)-isomer inhibited MAO-A from 83.3% to 90.8% and MAO-B from 82.7% to 92.3%. The (-)-isomer inhibited both forms of MAO much less than that observed with the (+)-isomer; levels of inhibition of MAO-A varied between 23.0% and 47.9%, while with MAO-B values were between 29.0% and 62.1%.

For *in vitro* MAO inhibition experiments, various concentrations of TCP isomers (10^{-4}-10^{-8} M) were incubated with brain homogenates to determine the extent of inhibition. The per cent inhibition was plotted against the log of the isomer concentration to determine the concentration required to inhibit MAO by 50% (IC_{50}). The results are reported in Table VIII. The stronger MAO-inhibiting properties of the (+)-isomer were even more apparent in the *in vitro* experiments. (+)-Tranylcypromine had an IC_{50} value almost 20x less than that of (-)-TCP for inhibition of MAO-A and almost 30x less than (-)-TCP for MAO-B. There was no significant difference in the extent of MAO inhibition between MAO-A and MAO-B for either isomer, although the difference approached the 0.05 level of significance with (+)-TCP.

3. Levels of Biogenic Amines in Brain after Administration of Tranylcypromine Isomers

Table VII. Per cent inhibition of monoamine oxidase *in vivo* by tranylcypromine isomers (1 mg/kg). Values represent the mean ± S.E.M.; N = 4-5.

	(-)-TCP		(+)-TCP	
	MAO-A	MAO-B	MAO-A	MAO-B
0.5 hr	23.0 (3.2)	29.0 (3.1)	83.3 (3.1)	86.6 (1.9)
1.5 hr	43.8 (3.6)	52.7 (2.7)	90.8 (2.1)	82.7 (2.1)
3.0 hr	47.9 (3.7)	62.1 (7.2)	84.4 (3.6)	92.3 (0.3)
6.0 hr	44.3 (4.5)	58.0 (3.6)	84.5 (2.7)	90.4 (1.1)

Table VIII. Inhibition of monoamine oxidase *in vitro* by tranylcypromine isomers. Values represent mean ± S.E.M.; N = 3.

	IC_{50} (M)	
Isomer	MAO-A	MAO-B
(+)-TCP	0.16 (0.12)	0.10 (0.04)
(-)-TCP	3.00 (0.40)	2.77 (0.29)

(a) β-Phenylethylamine

The brain levels of PEA after administration of TCP isomers are shown in Figure 20. The (+)-isomer had a much more dramatic effect on PEA levels than did the (-)-isomer. This effect is consistent with the greater MAO-inhibiting properties of the (+)-isomer. With (+)-TCP, the levels of PEA were significantly elevated above control values at all time intervals; however, there was no significant difference between the different time intervals.

The (-)-isomer caused no significant elevations of PEA above control values at any time interval. However, at the 6.0 hr interval, PEA was significantly depressed below control values ($p < 0.05$).

(b) 5-Hydroxytryptamine

The levels of 5-HT in the brain after treatment with TCP isomers is displayed in Figure 21. At 0.5 hr there was no increase in 5-HT levels in the (-)-TCP treated rats. However, by 1.5 hr and 6.0 hr, 5-HT levels were significantly elevated above controls. At the 3.0 hr time period, there was a slight drop from the previous (1.5 hr) time interval, and thus this group did not differ significantly from controls. There is no apparent reason for this drop.

The (+)-isomer also had a much more dramatic effect on 5-HT levels. Concentrations of 5-HT were significantly elevated above control values at all time intervals. At the 6.0 time interval, 5-HT levels were the highest, although they were not significantly different ($p > 0.05$) from the levels at 1.5 or 3.0 hr. At 6.0 hr, (+)-TCP caused a 3-fold increase above control values, compared to a 1.4-fold increase caused by (-)-TCP.

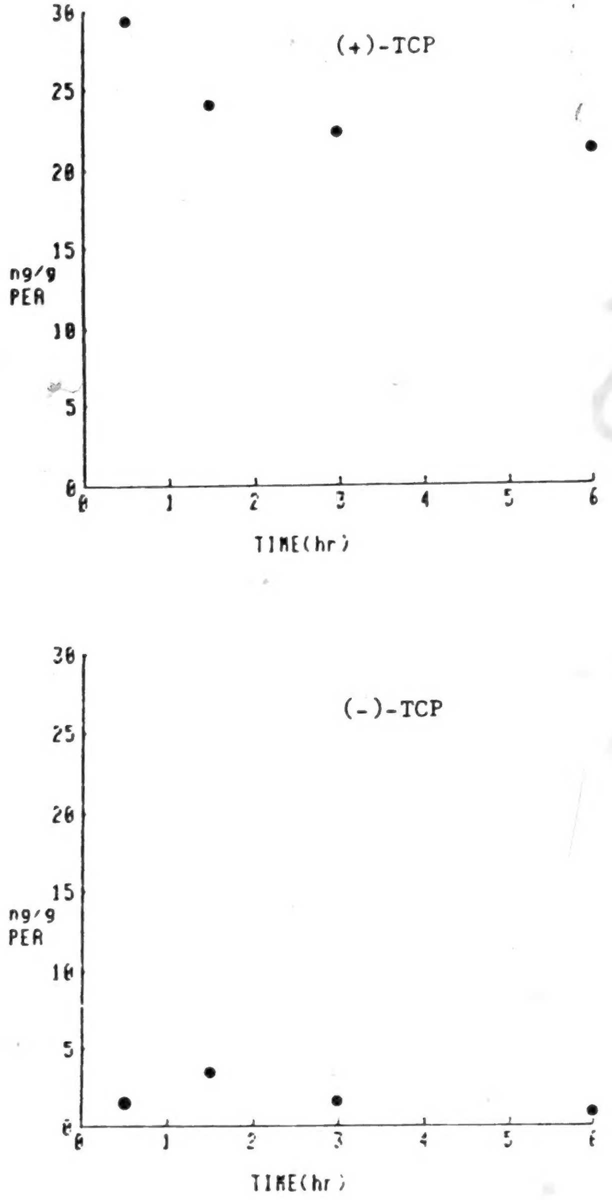


Figure 20. Levels of phenylethylamine in rat brain after dosing with tranylcypromine isomers. N=4-5.

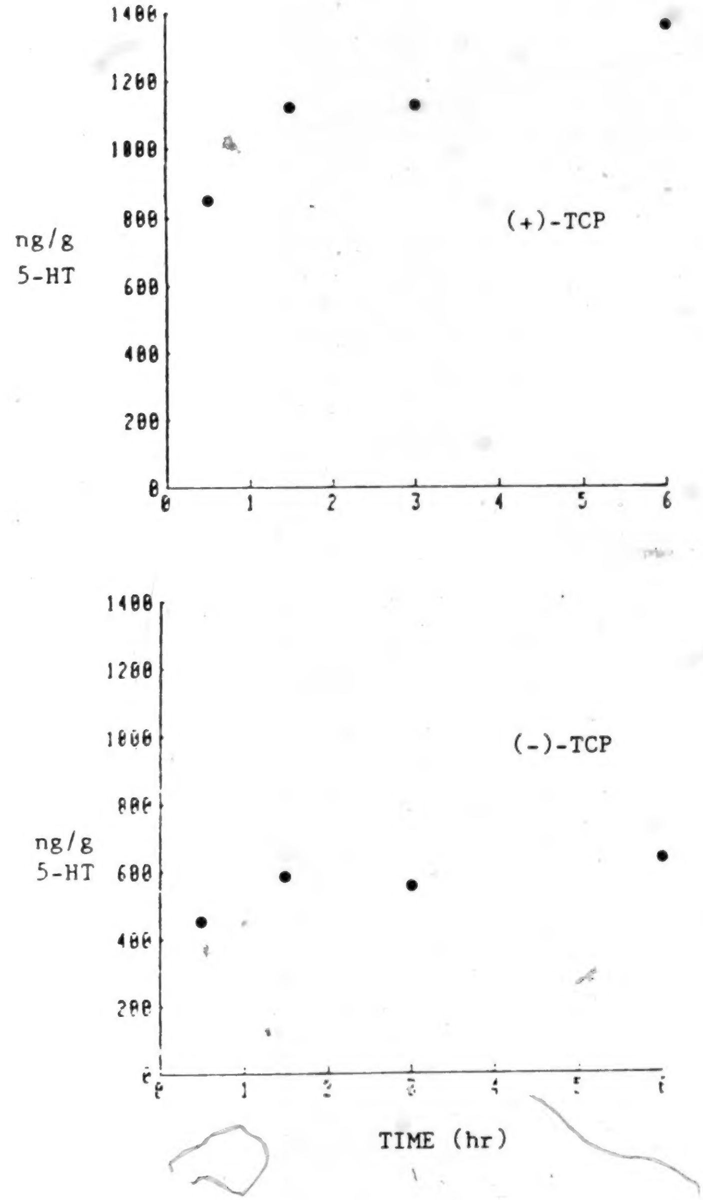


Figure 21. Levels of 5-HT in rat brain after dosing with tranylcypromine isomers. N=4-5.

4. The Effects of Tranylcypromine Isomers on the Inhibition of Reuptake of Tritiated Neurotransmitters

The effects of TCP isomers on the inhibition of reuptake of tritiated neurotransmitters into prisms prepared from brain regions are shown in Table IX. With all three neurotransmitters investigated, the (-)-isomer was a more potent inhibitor of reuptake than was the (+)-isomer. Both isomers appear to be particularly potent in inhibiting the reuptake of ^{3}H-NE. At 10^{-5} M drug concentration, the difference between the two isomers was not apparent, both isomers inhibiting ^{3}H-NE reuptake to approximately the same high extent. At 10^{-6} M drug concentration, however, the potency difference became apparent (72.9%, (-)-TCP; 59.2%, (+)-TCP). The difference between the isomers in inhibiting the reuptake of ^{3}H-DA and ^{3}H-5-HT was apparent at 10^{-5} M. The order of potency for inhibiting uptake of neurotransmitters for both isomers is: NE >> DA > 5-HT. The (-)-isomer's potency was equivalent to that of *p*-TA in inhibiting the reuptake of ^{3}H-NE, but less than *p*-TA in inhibiting reuptake of ^{3}H-DA and ^{3}H-5-HT. *Para*-tyramine is known to be a strong inhibitor of uptake of catecholamines and 5-HT (Raiteri *et al.*, 1977) and was used in these experiments as a reference compound.

5. The Effects of Tranylcypromine Isomers on the Release of Tritiated Neurotransmitters

The effects of TCP isomers on the release of ^{3}H-NE, ^{3}H-DA, and ^{3}H-5-HT from rat brain areas are shown in Table X. In contrast to the difference between the two isomers in inhibiting ^{3}H-NE reuptake at 10^{-6} M, little difference was observed in the ability of the two isomers to release ^{3}H-NE at 10^{-4} M or 10^{-5} M. It would be anticipated that at 10^{-6}

Table IX. The effects of tranylcypromine isomers on the inhibition of reuptake of tritiated neurotransmitters in prisms from rat brain. Values represent the mean of the per cent inhibition ± (S.E.M.). N = 4-10. Para-tyramine (p-TA) is included as a reference standard. Drug concentrations were 10^{-5} M for studies on ^{3}H-DA and ^{3}H-5-HT and 10^{-6} M for studies on ^{3}H-NE.

	(-)-TCP	(+)-TCP	pTA
^{3}H-NE (hypothalamus)	72.9 (1.6)	59.2 (1.9)	70.8 (0.81)
^{3}H-DA (striatum)	73.9 (1.3)	49.1 (3.5)	83.5 (0.86)
^{3}H-5-HT (striatum)	50.8 (1.7)	36.2 (4.0)	83.7 (2.8)

Table X. The effects of tranylcypromine isomers on the release of ^{3}H-norepinephrine, ^{3}H-dopamine, and ^{3}H-5-hydroxytryptamine from rat brain prisms. Values represent the mean per cent increase over control values ± (S.E.M.). N = 4-6.

	^{3}H-NE (hypothalamus)	^{3}H-DA (striatum)	^{3}H-5-HT (striatum)
(-)-TCP (10^{-4} M)	65 (19.1)	152 (8.9)	118 (10.2)
(+)-TCP (10^{-4} M)	51 (15.5)	53 (7.5)	32 (11.0)
(-)-TCP (10^{-5} M)	27 (5.9)	59 (6.4)	43 (9.1)
(+)-TCP (10^{-5} M)	34 (5.1)	11 (8.3)	13 (7.1)

M drug concentration (not tested), a concentration at which substantial ^{3}H-NE reuptake inhibition is apparent with both isomers, little release would occur. However, as with reuptake inhibition, the (-)-isomer is substantially more potent in releasing ^{3}H-DA and ^{3}H-5-HT.

III.C. Detection and Characterization of Para-Hydroxytranylcypromine as a Metabolite of Tranylcypromine

1. Detection and Analysis of Para-Hydroxytranylcypromine

The analytical method described in section II.F.2 was successfully applied to the analysis of *p*-OH-TCP in urine. The PFBZ derivative gave characteristic diagnostic ions by EI-MS and CI-MS (Figures 22 and 23, respectively). The average urinary excretion of *p*-OH-TCP from four rats was 1.33 μg/24 hr (± 0.07, S.E.M.). No *p*-OH-TCP could be deteced in untreated rats. To produce unequivocal evidence for the presence of *p*-OH-TCP in urine, SIM-MS was utilized. The quasimolecular ion (MH^+, m/z 538) in the CI-MS of derivatized *p*-OH-TCP was also the base peak in the spectrum and therefore was selected as the ion to be monitored by SIM. The CI-SIM mass spectra of an N,O-diPFB-*p*-OH-TCP standard, a control urine extract, and an extract of urine from a rat dosed with 1 mg/kg TCP are shown in Figure 24. A peak with the same retention time and m/z as derivatized *p*-OH-TCP was observed in the control urine. However, closer inspection of the spectrum utilizing the ANSWER mode of data analysis revealed that the interference actually eluted slightly before *p*-OH-TCP. Only in the TCP-treated rat urine was a peak observed that eluted at exactly the same time. These observations provide unequivocal evidence for the presence of *p*-OH-TCP in urine of TCP-

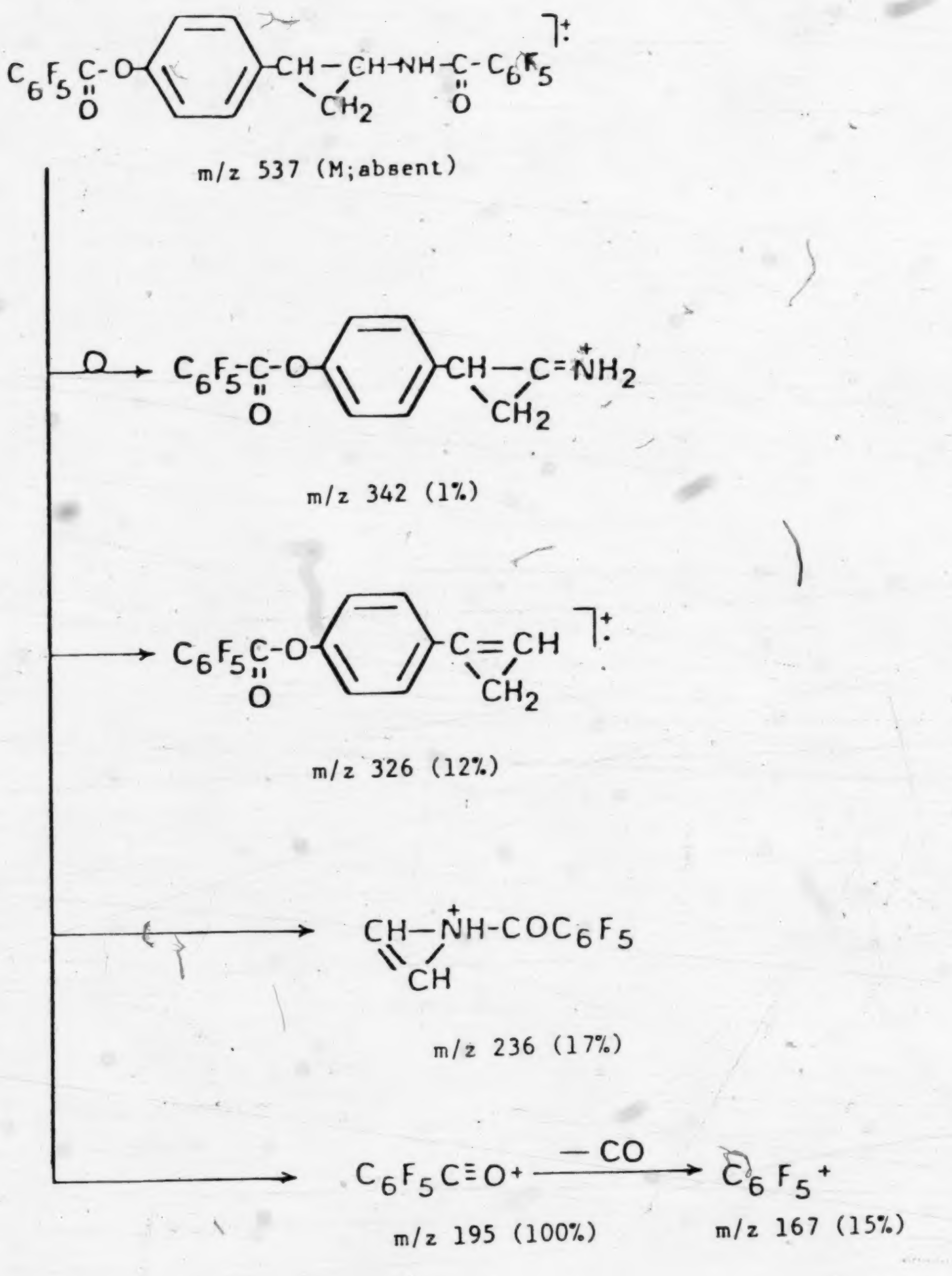

m/z 537 (M;absent)
m/z 342 (1%)
m/z 326 (12%)
CH—NH-COC6F5
m/z 236 (17%)
C6F5C≡O+
— CO
C6F5+
m/z 195 (100%)
m/z 167 (15%)

Figure 22. Fragmentation pattern of the electron-impact mass spectrum of the pentafluorobenzoyl derivative of para-hydroxytranylcypromine.

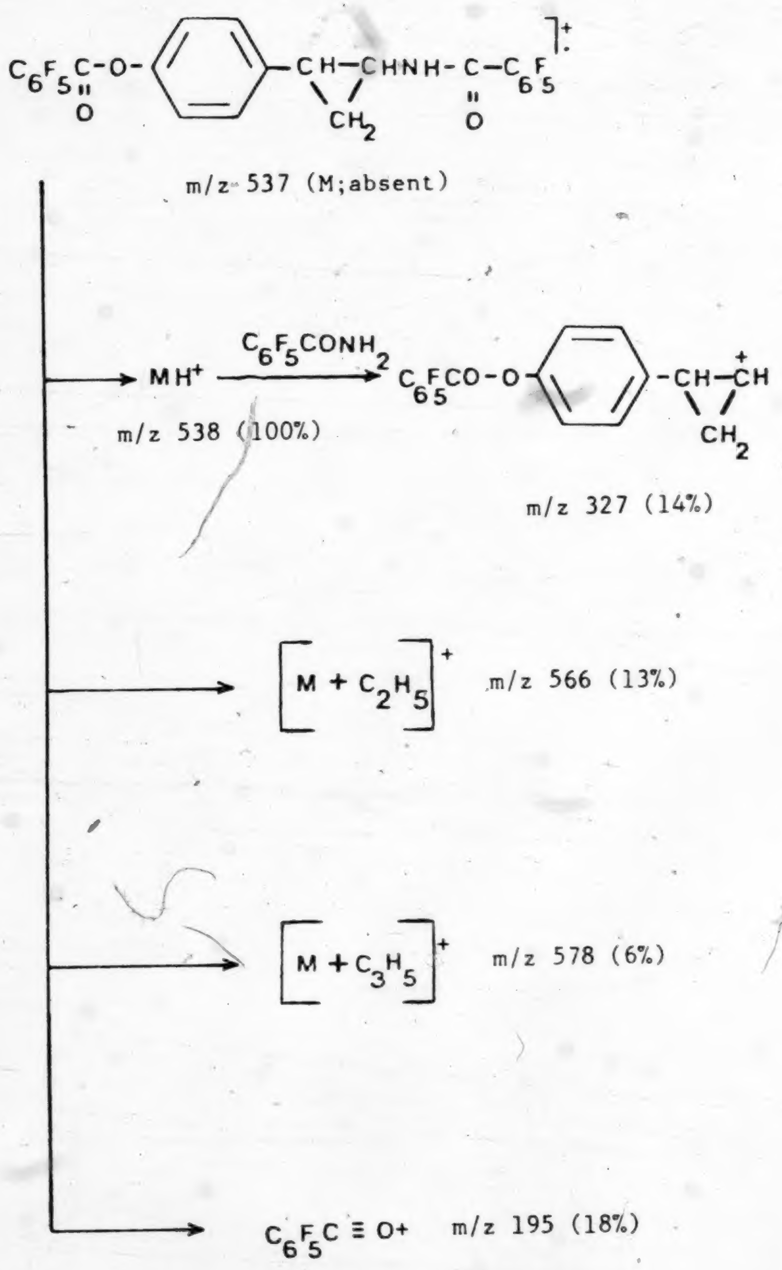

m/z 537 (M;absent)
MH+
$C_6F_5CONH_2$
m/z 538 (100%)
m/z 327 (14%)
$[M + C_2H_5]^+$
m/z 566 (13%)
$[M + C_3H_5]^+$
m/z 578 (6%)
$C_6F_5C \equiv O^+$
m/z 195 (18%)

Figure 23. Fragmentation pattern of the chemical ionization mass spectrum of the pentafluorobenzoyl derivative of para-hydroxytranylcypromine.

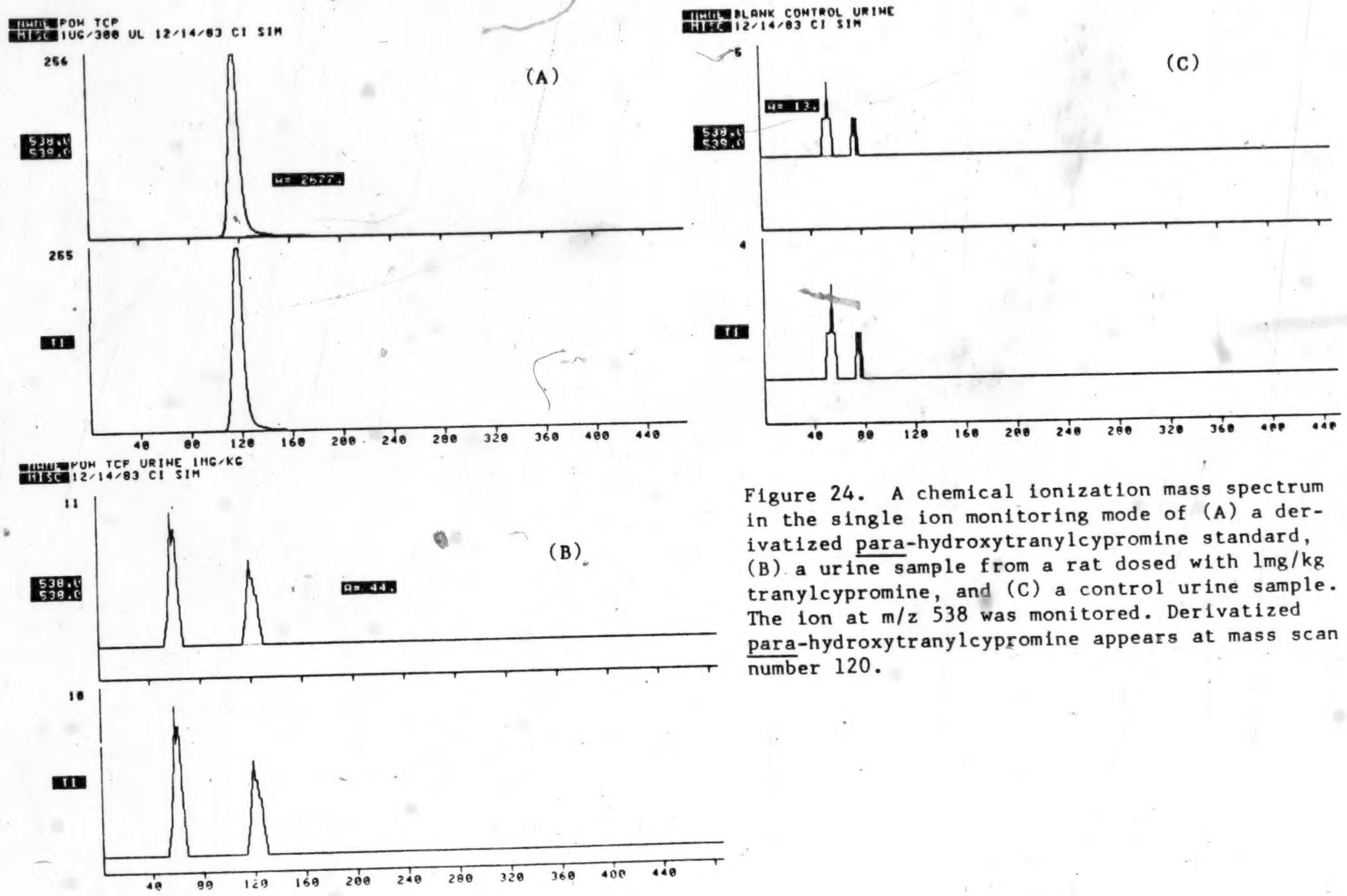


Figure 24. A chemical ionization mass spectrum in the single ion monitoring mode of (A) a derivatized para-hydroxytranylcypromine standard, (B) a urine sample from a rat dosed with 1mg/kg tranylcypromine, and (C) a control urine sample. The ion at m/z 538 was monitored. Derivatized para-hydroxytranylcypromine appears at mass scan number 120.

treated rats. Attempts to detect amphetamine as a metabolite of TCP in the brain using this procedure were unsuccessful.

Iprindole (10 mg/kg), an inhibitor of para-hydroxylation of AMP (Fuller and Hemrick-Luecke, 1980), is also apparently an inhibitor of para-hydroxylation of TCP, since pretreatment of rats with iprindole resulted in the disappearance of the p-OH-TCP peak in the chromatogram (Table XI) of a urine extract.

Using the procedure of Baker et al. (1982), p-OH-TCP could not be detected in brain 1.5 h after i.p. administration of TCP at a dose of 1 mg/kg. However, a large peak was noted in the GC trace after administration of TCP at a dose of 10 mg/kg. The structure of the compound represented by this peak was shown by EI-SIM-MS to be O-TFA, N-TFA, N-acetyl-para-hydroxytranylcypromine (see Figures 25 and 26).

In another series of experiments, the effects of pretreatment of rats with various psychotropic drugs on the levels of TCP and AMP were examined. It was anticipated that drugs that inhibit para-hydroxylation would cause an increase in the levels of parent drug (TCP or AMP). The results are presented in Table XII.

First, the effects of pretreatment with iprindole or saline on the brain levels of TCP or AMP were examined. Iprindole caused an increase in both TCP and AMP; however, the effect was more dramatic with AMP in that iprindole pretreatment caused more than a four-fold increase in brain AMP levels while approximately a two-fold increase in TCP was observed in iprindole-TCP-treated rat brains.

Pretreatments with the neuroleptics chlorpromazine and trifluperazine and the classic cytochrome P-450 inhibitor SKF 525A were also examined for their effects on brain TCP levels. Chlorpromazine displayed

Table XI. The effects of pretreatment with iprindole on urinary excretion of _para_-hydroxytranylcypromine. Iprindole (10 mg/kg) was injected i.p. 1 hr before a 1 mg/kg i.p. injection of tranylcypromine. Urine was collected for 24 hr after tranylcypromine administration, then frozen at -50°C until analysis. _Para_-hydroxytranylcypromine was quantitated by gas chromatography as described in section II.F.2. N = 4.

Pretreatment	Drug	p-OH-TCP (g/24 hr)
Saline	Saline	N.D.[a]
Saline	TCP	1.33 ± 0.07
Iprindole	TCP	N.D.[a]

[a] Not detected

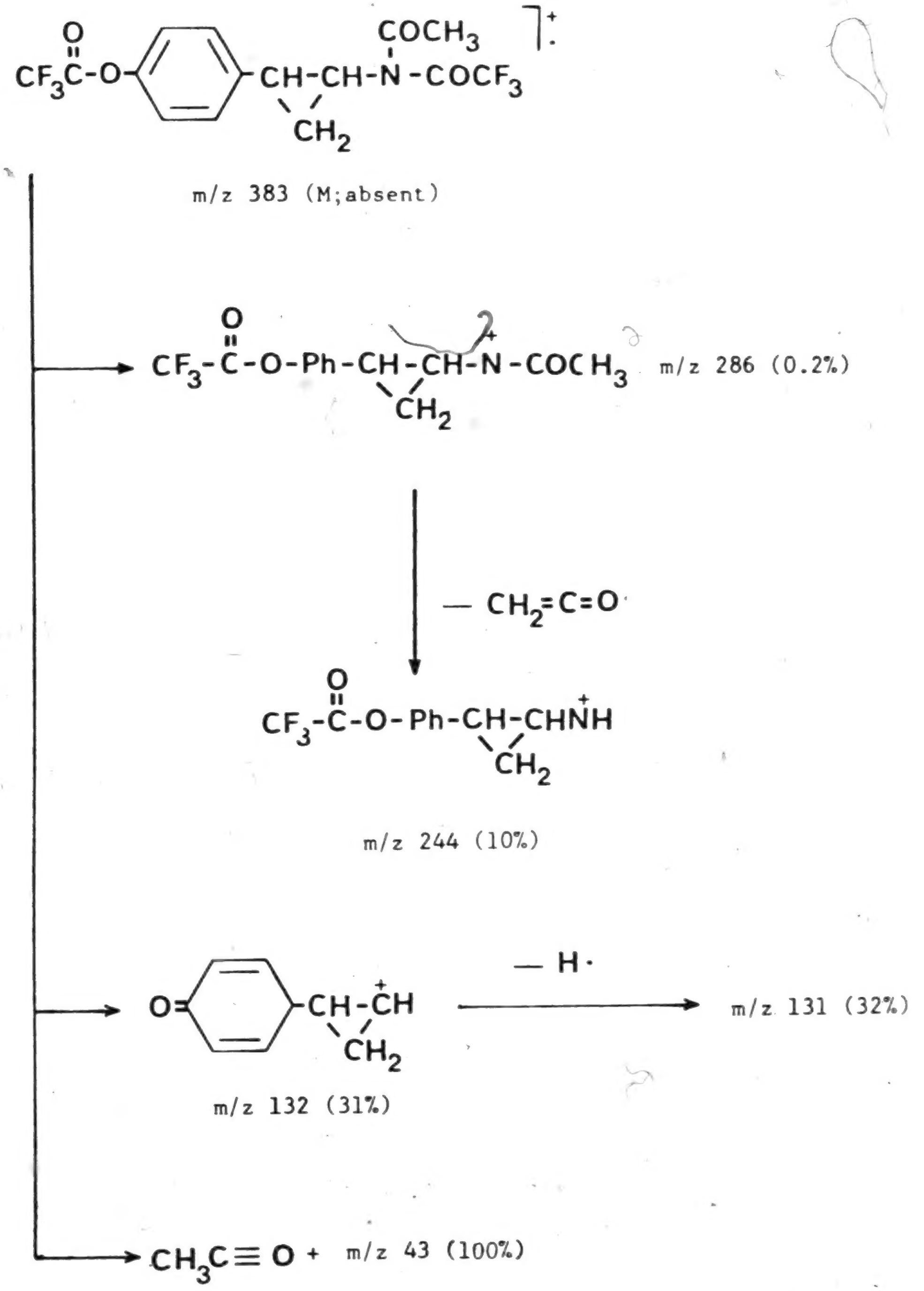

m/z 383 (M;absent)
m/z 286 (0.2%)
— CH2=C=O
m/z 244 (10%)
— H·
m/z 131 (32%)
m/z 132 (31%)
CH3C≡O +
m/z 43 (100%)

Figure 25. Fragmentation pattern of an electron-impact mass spectrum of O-trifluoroacetyl, N-trifluoroacetyl, N-acetyl-para-hydroxytranylcypromine.

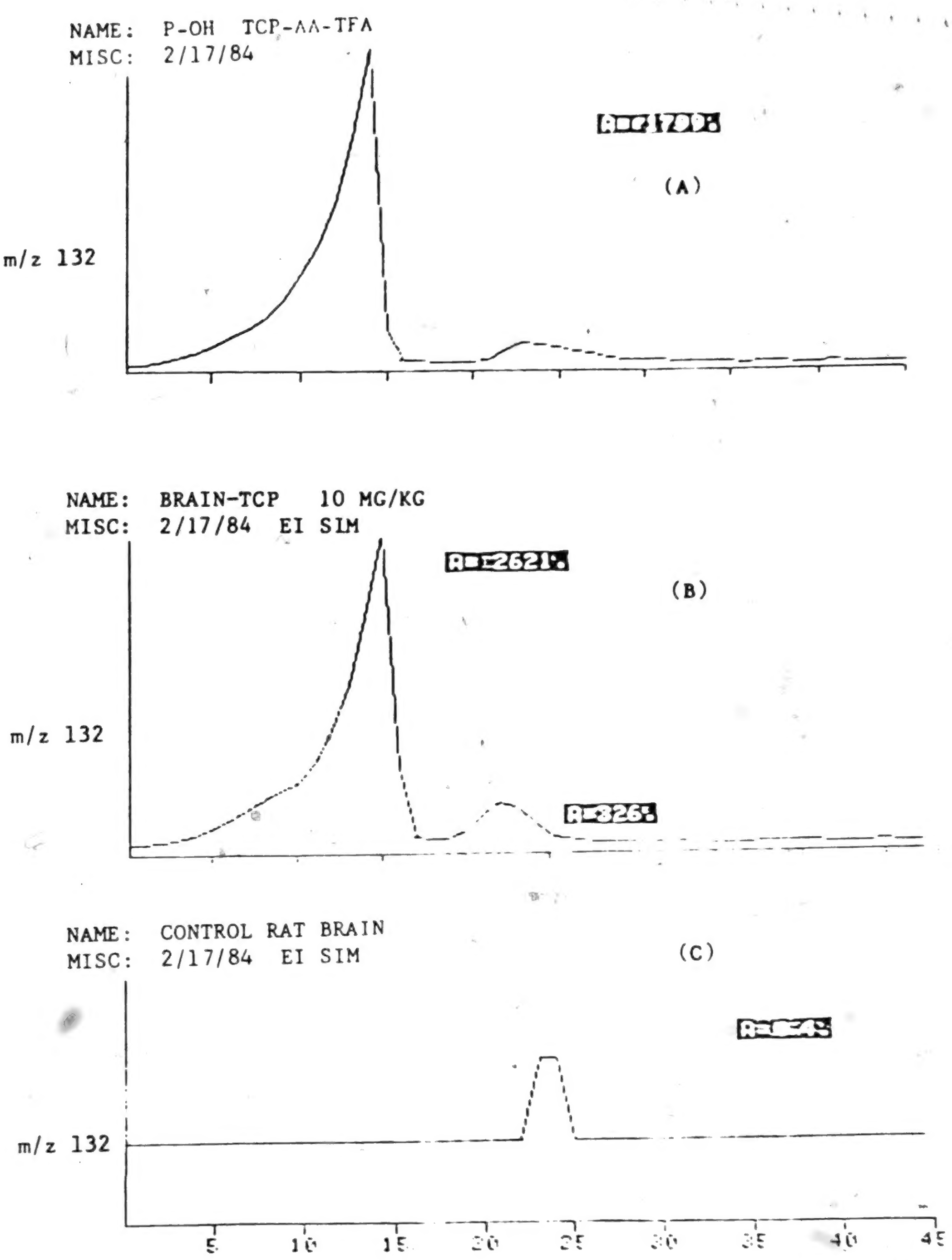


Figure 26. An electron-impact mass spectrum in the single ion monitoring mode of (A) a derivatized para-hydroxytranylcypromine standard, (B) a brain sample from a rat dosed with 10 mg/kg of tranylcypromine, and (C) a control rat brain.

Table XII. Effects of pretreatment with inhibitors of ring hydroxylation on brain concentrations of amphetamine and tranylcypromine in the rat.

Pretreatment[a]	Drug[b]	TCP[c]	AMP[c]	N
Saline	AMP		420 (41.9)	5
Iprindole	AMP		1977 (101.1)	5
Saline	TCP	258 (24.6)		5
Iprindole	TCP	502 (29.5)		5
Chlorpromazine	TCP	1004 (70.0)		4
Trifluperazine	TCP	730 (27.0)		4
SKF525A	TCP	639 (55.8)		4

[a] Pretreatments were administered at 3:00 p.m. Doses for all pretreatment drugs were 35 μmoles/kg (see Section II.B.3 for details).

[b] Drugs were administered at 4:00 p.m. at 1 mg/kg i.p. Animals were sacrificed 1.5 hours later.

[c] ng/g wet weight of brain tissue (± S.E.M.).

the most profound effect upon brain levels of TCP, increasing them almost four-fold above saline-pretreated animals.

These results verify that p-OH-TCP is a metabolite of TCP in rat brain and urine and that AMP could not be detected as a metabolite in the rat brain. In addition, it was demonstrated that a variety of psychotropic drugs and metabolic inhibitors can profoundly affect the levels of TCP in brain.

2. Biological Activity of Para-Hydroxytranylcypromine

(a) Inhibition of Monoamine Oxidase

Calculation of the IC_{50} values for the inhibition of brain MAO-A and MAO-B in vitro by TCP and p-OH-TCP showed that there was no significant difference between the two drugs (Table XIII). Both were potent inhibitors.

(b) Inhibition of Reuptake of Tritiated Neurotransmitters by Para-Hydroxytranylcypromine

Para-hydroxytranylcypromine was compared with TCP to determine if the hydroxylated metabolite retains the ability of the parent drug to inhibit reuptake of neurotransmitter amines. The results are shown in Table XIV. The reuptake-inhibiting properties of p-OH-TCP on ^{3}H-NE and ^{3}H-DA were very similar to those of TCP itself. Tranylcypromine and p-OH-TCP were more potent in inhibiting reuptake of ^{3}H-NE than of ^{3}H-DA or ^{3}H-5-HT. However, the TCP metabolite was considerably more potent than TCP in inhibiting ^{3}H-5-HT reuptake ($p < 0.001$). Thus, para-hydroxylation of TCP provided retention of the potent NE and DA reuptake inhibit-

Table XIII. Comparison of the *in vitro* inhibition of monoamine oxidase by tranylcypromine and *para*-hydroxytranylcypromine. Values represent the mean of the IC_{50} (M) ± S.E.M. N = 6.

	MAO-A	MAO-B
TCP	2.82 (0.28)	2.58 (0.27)
p-OH-TCP	2.66 (0.41)	2.82 (0.23)

Table XIV. Comparison of effects of para-hydroxytranylcypromine and tranylcypromine on the inhibition of reuptake of ^{3}H-dopamine, ^{3}H-norepinephrine, and ^{3}H-5-hydroxytryptamine in rat brain prisms. Values represent the mean of the per cent inhibition (± S.E.M.). N = 3-4. Drug concentrations were 10^{-5} M for DA and 5-HT experiments and 10^{-6} M for NE experiments.

	p-OH-TCP	TCP
^{3}H-NE (hypothalamus)	63.8 (5.0)	75.1 (1.9)
^{3}H-DA (striatum)	67.4 (0.9)	70.9 (1.8)
^{3}H-5-HT (striatum)	76.6 (1.4)	28.8 (0.2)

ing properties of the parent drug, while actually increasing the ability to inhibit 5-HT reuptake.

(c) Release of Tritiated Neurotransmitters by Para-Hydroxytranylcypromine

The ability of p-OH-TCP to release ^{3}H-NE, ^{3}H-DA, and ^{3}H-5-HT from rat brain tissue was compared with TCP. The results are reported in Table XV. At 10^{-4} M, p-OH-TCP was essentially equipotent with TCP in releasing ^{3}H-NE from prisms of hypothalamic tissue. Approximately the same degree of release was seen with ^{3}H-DA (38-41% above control values), and again the hydroxylated metabolite was equipotent with TCP. However, a different pattern of release was seen with ^{3}H-5-HT in striatal tissue. Para-hydroxytranylcypromine was a much more potent releaser of ^{3}H-5-HT than was TCP itself ($p < 0.001$).

III.D. Detection of 6-Hydroxytetrahydro-β-Carboline

The method developed for the extraction and analysis of 6-OH-THBC in rat urine employed a double-derivatization procedure as described in Materials and Methods, Section II.F.3. The structure of N-PFP,O-PFBZ-6-OH-THBC was confirmed by EI-MS (Figure 27). A molecular ion at m/z 528 (25% relative abundance) was present in addition to several other diagnostic ions. These ions were utilized to demonstrate the unequivocal presence of 6-OH-THBC in the urine of a TCP-5-HTP-treated rat by EI-SIM mass spectrometry.

Figure 28 depicts an EI-SIM mass spectrum from an N-PFP,O-PFBZ-6-OH-THBC standard, a urine sample from a rat dosed with TCP and 5-HTP, and a control urine. The molecular ion (m/z 528) and two other characteristic ions (m/z 158 and m/z 333) were monitored simultaneously. In

Table XV. Comparison of the effects of para-hydroxytranylcypromine and tranylcypromine on release of ^{3}H-norepinephrine, ^{3}H-dopamine, and ^{3}H-5-hydroxytryptamine from rat brain prisms. Values represent the mean of the per cent increase of release over control values (± S.E.M.). N = 5-10. Drug concentrations were 10^{-5} M for DA and 5-HT experiments and 10^{-4} M for NE experiments.

	p-OH-TCP	TCP
^{3}H-NE (hypothalamus)	42.6 (9.6)	36.1 (6.1)
^{3}H-DA (striatum)	38.4 (5.3)	40.9 (8.2)
^{3}H-5-HT (striatum)	71.3 (7.6)[a]	27.9 (5.5)

[a] Significantly different from TCP ($p < 0.001$).

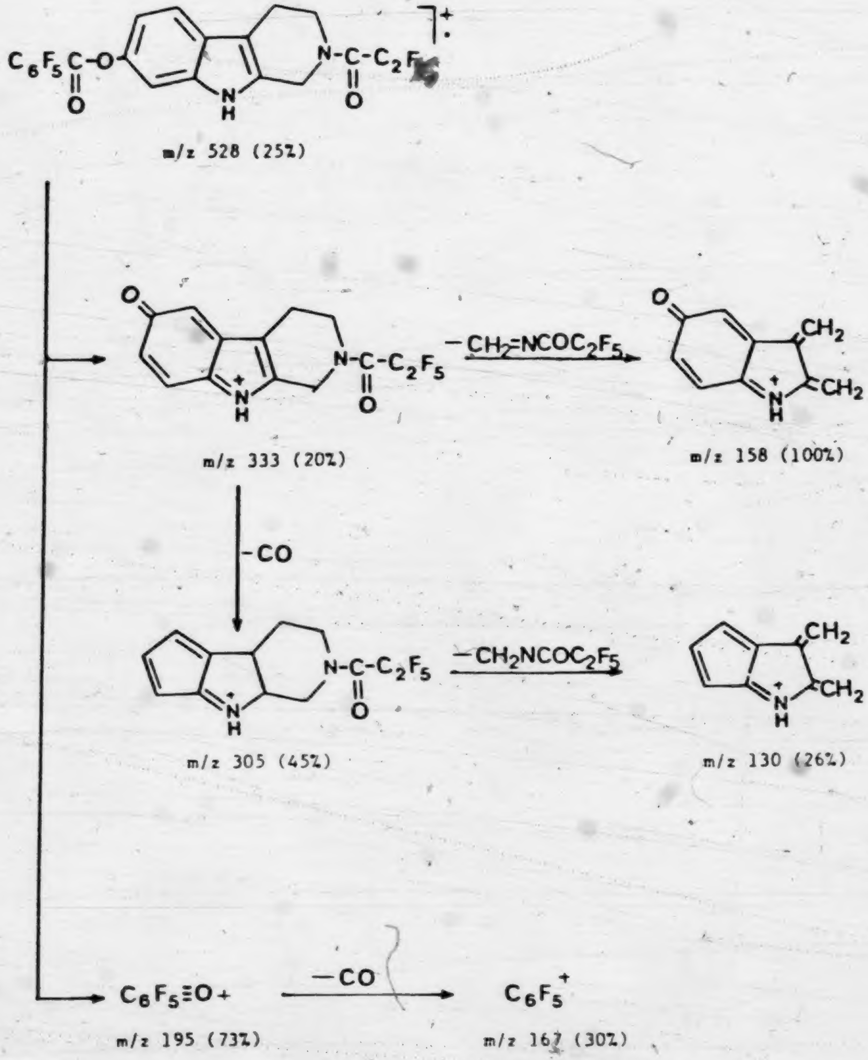

C6F5C-O
N-C-C2F5
m/z 528 (25%)
N-C-C2F5
m/z 333 (20%)
−CH2=NCOC2F5
CH2
CH2
m/z 158 (100%)
−CO
N-C-C2F5
m/z 305 (45%)
−CH2NCOC2F5
CH2
CH2
m/z 130 (26%)
C6F5≡O+
m/z 195 (73%)
−CO
C6F5+
m/z 167 (30%)

Figure 27. Fragmentation pattern of the electron-impact mass spectrum of derivatized 6-hydroxytetrahydro-β-carboline.

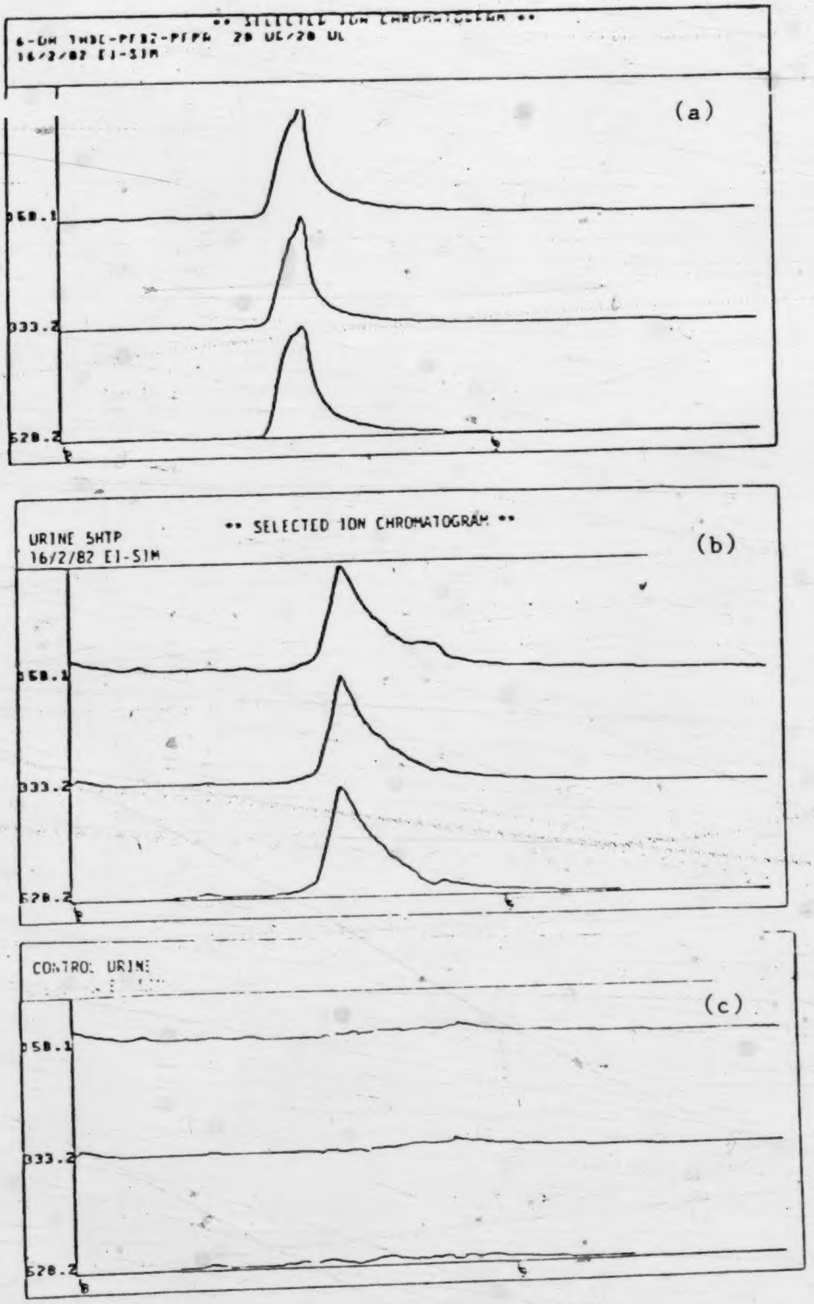


Figure 28. An electron-impact mass spectrum in the selected ion monitoring mode of (a) a derivatized standard of 6-hydroxytetrahydro-β-carboline, (b) a urine sample from a rat dosed with tranylcypromine and 5-hydroxytryptophan, and (c) a control urine sample.

the TCP + 5-HTP-treated rat urine, a peak eluting from the GC column at exactly the same retention time and containing all 3 diagnostic ions was observed. None of these ions was observed in urine from control rats at this retention time. The coinciding of the 3 characteristic ions eluting at exactly the same retention time as an authentic standard of derivatized 6-OH-THBC provides strong evidence for the detection of 6-OH-THBC.

These results were also confirmed by analysis of urine samples using GC. However, the presence of 6-OH-THBC as assessed by GC was not consistent. It is possible that the inconsistent detection of 6-OH-THBC may have been due to problems in reproducibility of the method since the standard curves constructed were often not linear. Therefore, it may be that the failure to detect 6-OH-THBC consistently in urine samples was due to anomalies inherent in the method. Numerous attempts to determine the cause of irreproducibility and non-linearity of standards were not successful. In light of these problems, further attempts to quantitate 6-OH-THBC in urine were not made.

DISCUSSION

IV. DISCUSSION

IV.A. Acute and Chronic Treatment with Tranylcypromine

1. Anorexiant Effects, Brain Tranylcypromine Concentrations, and Monoamine Oxidase Activity

Although the absolute weights of chronic TCP-treated rats did not differ significantly from the saline-treated rats, the amount of weight gained by the drug-treated group was significantly less. This observation is consistent with the results reported by Willis and Smith (1982) who studied the anorexic properties of nialamide, pargyline, and TCP. They studied mean change in body weight, mean quantity of food eaten, and mean volume of water consumed within 24 hrs after i.p. injection of four different doses of each of the MAOIs mentioned above. A summary of their results is shown in Table XVI. All three MAOIs significantly reduced ($p < 0.001$) body weight, food consumption, and water intake at the 3 highest doses tested. Tranylcypromine at 1 mg/kg, nialamide at 20 mg/kg, and pargyline at 15 mg/kg did not significantly reduce these parameters over the first 24 hrs after i.p. injection. It should be noted that there appears to be a good dose-response relationship with all three parameters studied. It was also reported that the mean number of days required for pargyline-treated rats to regain their preinjection body weight after stopping pargyline was 1.3 days for a 10 mg/kg dose and 3.6 days for a 50 mg/kg dose.

Thus, although a 1 mg/kg dose of TCP does not cause body weight decreases within the first 24 hrs after administration, the present study has shown that within three weeks of continuous daily administration of a clinically relevant dose, decreases in weight gain are appar-

Table XVI. A condensed summary of the results of Willis and Smith (1982). Nialamide and pargyline produced results similar to those obtained with tranylcypromine.

A. The mean change in rat body weight over 24 hours after intraperitoneal injection of various doses of tranylcypromine.

Treatment	Dose (mg/kg)	Change in Body Weight (grams)
Control	--	+2
Tranylcypromine	1	+1
Tranylcypromine	2.5	-5[a]
Tranylcypromine	5.0	-6[a]
Tranylcypromine	10.0	-22[a]

B. The mean quantity of food eaten in the 24 hours after intraperitoneal injection of various doses of tranylcypromine.

Treatment	Dose	Grams of Food Eaten
Control	--	25
Tranylcypromine	1.0	22
Tranylcypromine	2.5	11[a]
Tranylcypromine	5.0	9[a]
Tranylcypromine	10.0	3[a]

[a] Significantly different from control values ($p < 0.001$).

ent. The results of Willis and Smith (1982) reveal that this effect is probably caused, at least in part, by a true anorexiogenic effect, that is, the decreased weight gain is likely caused by decreased food consumption. This conclusion is also supported by the work of Leibowitz and Rossakis (1978, 1979) who have studied the effects of hypothalamic injections of catecholamines on feeding behavior. Their extensive studies have shown that in food-deprived, pargyline-treated rats, injections of DA and epinephrine into the perfornical region of the hypothalamus potently suppress food intake. These effects were apparent at doses as low as 31 ng of DA or 150 ng epinephrine. This effect was reliably potentiated by pretreatment with either catecholamine reuptake inhibitors (desipramine and benztropine) or MAOIs (pargyline). Pargyline had a particularly strong potentiating action (100% increase in responsiveness over non-pargyline pretreated animals) with DA, indicating the high susceptibility of the amine to catabolism by MAO.

Perfornical injections of catecholamine agonists such as AMP, mazindol, and phenmetrazine also produced feeding suppression, and this effect was positively correlated with dose. It was concluded that perfornical hypothalamic catecholamine neurons, through DA receptors and β-adrenoceptors, are involved in inhibiting feeding behavior, as well as mediating the anorexiogenic properties of AMP, mazindol, and MAOIs.

Tranylcypromine appeared to enter the brain readily and accumulate in brain tissue after continuous daily treatments. As outlined in Section I.C.7, a daily dose of 1 mg/kg is comparable to a clinical situation. Considering the higher rate of metabolism found in rodents in general compared to humans, this dose could be considered conservative. The concentration of TCP in the brain approximately doubled between the first and 21st days of treatment. After 21 days, TCP accumulation appeared to be approaching saturation since little additional drug accumulated between days 21 and 42. If indeed humans do have a slower rate of metabolism, it is conceivable that the saturation level of TCP in the human brain is less than 21 days; however, this may be offset by other factors, for example, the larger relative size and capacity of the human versus rodent brain. In light of these and other factors that determine drug pharmacodynamics, and without further experimental evidence, projections of saturation times in human brain tissue are unwarranted.

Although drug clearance after termination of treatment was not examined in the present study, Bieck and Antonin (1982) estimated the half-life of TCP clearance in humans by measuring the decrease in the b.p. response to intravenous tyramine after discontinuation of long-term (4 weeks) TCP treatment. They concluded that the elimination of TCP occurs in two distinct phases: a fast initial phase with an estimated pharmacodynamic half-life of 1-2 days, followed by a slower elimination phase with a half-life of 2-3 weeks.

Thus, TCP accumulation and elimination after long-term dosing are biphasic. The drug enters the brain rapidly after the first few doses and accumulates slowly to a steady-state level. Conversely, after cessation of dosing, the drug levels may drop rapidly at first, followed by a slower second phase of elimination. Although the pressor response measurement may or may not be a good indicator of brain TCP levels, Bieck and Antonin (1982) also reported that urinary excretion of T correlated with the rise and fall of the pressor response at the initiation and cessation of TCP treatment. Therefore, it appears that the pressor response (and T excretion; see Section IV.A.2) may be an indication of MAO inhibition.

In the study reported here, the extent of MAO inhibition in the brain was also measured during chronic TCP treatment. A single dose of 1 mg/kg inhibited both forms of the enzyme by approximately 87%. This is consistent with the finding of Simpson et al. (1983) who reported a single 10 mg dose of TCP inhibited human platelet MAO activity by almost 80%. This dose of TCP would be (on a mg/kg basis) proportionately even less than that used in the present study. Together, these results indicate that clinical and even subclinical doses of TCP given acutely inhibit MAO activity enough (80-90%) to produce a level of enzyme inhibition that is within the therapeutic range (Robinson et al., 1978) within the first day of treatment. This range of enzyme inhibition is also sufficient to elevate amine levels (see below).

Robinson et al. (1979) reported that 7.5 mg/kg phenelzine and 5 mg/kg TCP inhibited rat brain MAO activity (as assessed by using C^{14}-tyramine as substrate) by 95-98% within the first week of dosing (single daily i.p. dose). They also reported that this was the maximum degree of inhibition obtained after chronic (21-day) treatment with these doses. Therefore, even with a dose 5x higher than that used in the present study, the level of enzyme inhibition rarely reaches 100%. Similar results were obtained in the present study, where even after 42 days of TCP treatment, the average level of enzyme inhibition was 95.4% and 92.7% for MAO-A and MAO-B respectively. In this regard, it is interesting to note that Shekhar et al. (1982) have recently described a clorgyline- and deprenyl-insensitive form of MAO in rat brain. This MAO activity was found in the soluble fraction of rat brain homogenates. The enzyme was not inhibited in vivo by high doses of clorgyline or deprenyl

(12 mg/kg for 2 hrs). In contrast to the mitochondrial forms of the enzyme, MAO activity associated with the soluble fraction did not show the usual biphasic dose-response kinetics with clorgyline when kynuramine was used as substrate. They speculated that "the ineffectiveness of some MAOIs may be attributed to the fact that these compounds may not be inhibiting the enzyme totally, i.e. both the mitochondrial and soluble forms, thereby causing only partial inhibition." They further suggested that the process of mitochondrial inhibition may be mediated through specific receptors which are different from those present in the soluble fraction. If this were so, it would indicate that the soluble form is not simply another form or isozyme of the mitochondrial MAO-A and MAO-B forms, but a completely different enzyme.

Another observation that was noted in the present study was the lack of specificity of TCP between MAO-A and MAO-B after acute or chronic administration. Although TCP is generally considered a non-specific inhibitor (Knoll, 1980), several groups have reported that TCP is more selective towards MAO-B (Collins and Sandler, 1971; Donnelly and Murphy, 1977). In the present study, both forms were inhibited to nearly the same extent (within 2.7%) of one another throughout the chronic treatment. In fact, at the end of the chronic TCP treatment (42 days), MAO-A was inhibited to a slightly greater extent, although at no time period did these differences reach statistical significance.

One would expect that if TCP was selective, it would be most selective after a single low dose (as in day 1 of the present study) since it has been suggested that long-term treatment with the selective inhibitors clorgyline and deprenyl decreases selectivity (Waldmeier and Felner, 1978; Egashira et al., 1976), particularly if higher doses are utilized. In addition, the immediate and sustained increases of both the MAO-A specific substrate 5-HT and the MAO-B specific substrate PEA in the brain and urine are further proof of the nonselective properties of TCP.

2. Effects of Acute and Chronic Tranylcypromine Treatment on Levels of Biogenic Amines

The response of the trace amines to chronically administered low doses of TCP has not been studied previously. The results obtained in the present study indicate that there are differences in the response between PEA and T and between the responses in the brain levels and urinary excretion of each individual amine.

Brain concentrations of PEA increased to 19x those of control values after a single dose of 1 mg/kg of TCP. This level was maintained throughout the 42-day dosing period. The urinary excretion of PEA followed a different course; peak levels were not reached on the first day, but were attained by the second time interval (10 days) studied. It is possible that, due to the lipophilic nature of TCP and its rapid entry into the brain, brain MAO is initially and preferentially inhibited over MAO located in other organs, for example, liver and kidney. It is known that brain tissue contains higher concentrations of lipids than liver or kidney (McIlwain and Bachelard, 1971).

The primary route of formation of PEA in the body is via decarboxylation of phenylalanine by amino acid decarboxylase (AAD) (Huebert, 1980). Considering the ubiquitous distribution of AAD and phenylalanine, PEA is probably formed in many organs of the body, although very low levels are maintained by catabolism by MAO. All these sources would contribute to urinary excretion of PEA. Karoum et al. (1979) reported that there was no diurnal variation in urinary excretion of PEA (or _m_- and _p_-tyramine), and that ingestion of 200 grams of chocolate containing 1 mg PEA and 10 mg of _p_-tyramine did not significantly alter urinary excretion of these amines. They concluded that PEA and _p_-tyramine were produced from endogenous sources and that the direct contribution of the diet to urine excretion was small. A similar case exists for T, which is formed by the action of AAD on tryptophan. In fact, according to Rodnight (1961), T in human urine is derived almost entirely from metabolism in the kidney. Bieck and Antonin (1982) noted that the excretion of T in human urine became significantly elevated after the cumulative oral dose of TCP exceeded 0.6 mg/kg. Their interest in studying T excretion stemmed from the possibility of using this parameter as a simple, noninvasive measurement of MAO inhibition, particularly as an alternative to platelet MAO measurements, which are invasive and reveal no direct information as to the degree of MAO-A inhibition. The present results are consistent with those of Bieck and Antonin (1982) in that an immediate increase in T excretion was seen in the rat after a comparable dose of TCP.

Within the rat brain, the highest levels of both PEA and T are present in the striatum (Durden _et_ _al._, 1973; Philips _et_ _al._, 1974) and in the hypothalamus (Karoum _et_ _al._, 1979). Philips _et_ _al._ (1978) reported that the distribution of the trace amines in human brain is similar to that observed in the rat brain. Tryptamine appears to be most concentrated in the basal ganglia, while PEA, although present in relatively large amounts in the basal ganglia, is more evenly distributed throughout the brain. It was also suggested that the relatively homogenous distribution of PEA may be due to the greater membrane permeability of this amine compared to other biogenic amines.

The results of having increased levels of these biogenic amines maintained over a long-term drug regimen, such as is typical with MAOIs, are unknown. The brain subregions mentioned above are intimately related to motor output (striatum) and to appetite, sleep, and sexual activities (hypothalamus). Monoamine oxidase inhibitors are known to have effects on all of these parameters. It is possible that the trace amines may operate to amplify these effects, and hence their appellation as neuromodulators (Boulton, 1976; Boulton, 1980).

Brain levels of 5-HT increased after a single injection of TCP and continued to rise throughout the first 21 days. By this time a plateau had been reached. As stated above, brain 5-HT levels correlated highly with drug concentration throughout the entire long-term dosing period. 5-Hydroxytryptamine concentrations did not correlate as well with MAO-A inhibition ($r = 0.8003$, nonsignificant $p > 0.05$). This lack of significant correlation could be due to the fact that brain 5-HT concentrations varied over a wide range, while MAO inhibition varied over a very narrow range; hence a good correlation would be difficult to show.

The trend observed in 5-HT levels over the course of long-term TCP treatment is not in accordance with that reported by Robinson et al. (1979). In their study, rats were treated daily with a single i.p. dose of 5 mg/kg of TCP or 7.5 mg/kg or 15 mg/kg of phenelzine for up to 42 days. They report that "for all drug treatments, 5-HT levels were maximum at 7 days, with increases ranging from 3-fold with low-dose phenelzine administration to 8- to 10-fold increases with high dose phenelzine and TCP. With low dose phenelzine and TCP, 5-HT levels had returned nearly to control levels by 14 days of treatment, while with high dose phenelzine there was a more gradual decline over six weeks." Campbell et al. (1979) obtained similar results using clorgyline at high (1 mg/kg) and low doses (0.5 mg/kg). They observed minimal changes in 5-HT levels at the high clorgyline dose after a single dose, but after 14 days of administration (single daily i.p. injection) both high and low doses of clorgyline produced significant (2-fold) increases. However, they reported that within the following 7 days (21 days of dosing) 5-HT levels had returned to control values. Two doses of pargyline (4 mg/kg/day and 1 mg/kg/day) were also administered for 21 days. With the higher dose of pargyline, 5-HT levels were significantly elevated at 21 days, while the low dose produced a significantly lower 5-HT concentration compared to controls at 21 days. It was proposed that the loss of specificity with 4 mg/kg/day dose of pargyline produced increased 5-HT levels after chronic dosing, while no explanation was given for a significant decrease in the low-dose pargyline group.

Robinson et al. (1979) suggested that adaptive changes in tryptophan hydroxylase activity may occur during the declining phase of 5-HT concentrations. However, they found no change in the activity of this enzyme over the course of long-term TCP treatment. Campbell et al. (1979) also reported no change in tryptophan hydroxylase activity after long-term treatment with clorgyline or pargyline.

However, Robinson et al. (1979) did report an increase in the activity of AAD. This appears to be a paradoxical finding by these researchers since they report a decline in 5-HT concentrations after 1 week. An increase in AAD would be compatible with the results obtained in my study, particularly since increased tryptophan availability has been reported to increase 5-HT levels in the CNS (Warsh et al., 1979).

Tabakoff and Moses (1976) reported that TCP raises tryptophan levels in mouse brain. Surprisingly, pargyline given at a dose which increases brain 5-HT to approximately the same level as in the TCP-treated mice had no effect on tryptophan levels. Considering this effect, and the fact that TCP, but not phenelzine, produced an increase in AAD activity in the study by Robinson *et al.* (1979), one could speculate that the induction of AAD and possibly an elevation in tryptophan may be a selective effect of TCP. Thus, in the studies of Robinson *et al.* (1979) and Campbell *et al.* (1979) it is unclear why 5-HT levels returned to baseline values, considering that (1) MAO was inhibited greater than 95% throughout the chronic treatment periods, (2) there was no change in tryptophan hydroxylase activity, and (3) TCP induced a paradoxical increase in AAD and possibly in tryptophan availability after long-term treatment.

Although the dose of TCP used in the present study was lower than that of Robinson *et al.* (1979) (1 mg/kg vs. 5 mg/kg), it is unlikely that this was a factor in the discrepancy. It could be hypothesized that by using a smaller dose the time of the decline in 5-HT may be delayed. However, two observations argue against this: (1) no decline in 5-HT concentrations was apparent in the present study, even after 42 days of dosing, and (2) the results of the high and low dose phenelzine experiments of Robinson *et al.* (1979) indicate that lower doses of MAOIs may expedite this trend rather than delay it, i.e. the return of 5-HT levels to control values occurred earlier in the low-dose phenelzine group compared with the high-dose phenelzine group.

Another anomaly between the present study and that of Robinson *et al.* (1979) was the difference in the maximum 5-HT values observed. In my study, maximum 5-HT concentrations (at 42 days) were 1081 ng/g (2.4x control values) while those of Robinson et al. (1979) were approximately 3500 ng/g (control values not reported). Although the dose in the latter study was higher, similar studies utilizing a dose even higher (10 mg/kg) than that of Robinson et al. (5 mg/kg) and an identical time of sacrifice after injection (6 hr) indicate a maximum 5-HT level of 2500 ng/g after 19 days of dosing (Baker, LeGatt, and Coutts, unpublished observations).

Two other studies have examined the effects of long-term administration of MAOIs on brain 5-HT levels. Waldmeier et al. (1981) examined the effects of chronic low dose (0.3 mg/kg) clorgyline treatment on biogenic amines in rat brain subregions. They stated that "noradrenaline and serotonin levels were markedly and simultaneously increased with a concomitant small decrease in biosynthesis. In no case did we find a reversal of these effects in the course of 21 days' treatment." They also stated that "clorgyline and other irreversible inhibitors have similar effects on MAO activity in the whole brain and in areas like the corpus striatum, hypothalamus, cortex and brain stem." Although regulation of the synthesis and utilization of biogenic amines may differ from one area to another, it appears from additional studies done by this group that, despite a moderate decrease in tryptophan hydroxylase activity in all subregions, increases in 5-HT levels were uniform throughout the different brain subregions studied.

Savage et al. (1980) examined the effects of chronic MAOIs and 5-HT reuptake inhibitors on brain 5-HT levels and on ^{3}H-5-HT binding sites in rat brain. They administered the MAOIs nialamide, clorgyline, deprenyl, pargyline, or TCP or the 5-HT reuptake inhibitors amitriptyline, chlorimipramine, or fluoxetine to rats for up to 16 days. Treatment with inhibitors of 5-HT reuptake did not change the specific binding of ^{3}H-5-HT in either cerebral cortex or hippocampus, nor did they produce any consistent alterations in 5-HT concentrations. In contrast, MAOIs capable of inhibiting MAO-A or nonselective MAOIs significantly decreased ^{3}H-5-HT binding after both 4 and 16 days of treatment. These effects were not observed after 1-3 days of dosing. The reduction in 5-HT binding sites was due to a decrease in the maximum number of binding sites with no change in affinity of the binding site for 5-HT. In addition, MAOIs caused significant elevations in brain 5-HT levels at all time intervals (except for nialamide-treated rats, specific 5-HT concentrations were not reported).

The profile of 5-HT in the brain in response to chronic TCP treatment observed in the present study coincides with the profile observed with the brain levels of the drug. The plateauing point for both of these is approximately 15-20 days, a time which corresponds to the typical time required for the onset of clinical efficacy to become apparent. Robinson et al. (1973) have reported that a group of phenelzine-treated patients reached a therapeutic plateau by the fourth week of treatment; 60% of the ultimate clinical improvement in depressive symptoms occurred by the end of the second treatment week. Minimal changes were seen between 4-6 weeks of treatment.

Thus it appears that during chronic low-dose TCP administration, brain drug levels, brain 5-HT levels, and the reported onset of clinical improvement all culminate at approximately the same time (10-15 days after initiation of treatment).

IV.B. Administration of Tranylcypromine Isomers

1. Disposition of, and Monoamine Oxidase Inhibition by, Tranylcypromine Isomers

There are substantial differences in the disposition of the (+)- and (-)-isomers of TCP in rat brain; (-)-TCP enters the brain faster, attains a higher peak concentration, and is subsequently cleared faster than (+)-TCP. The half-life of (+)-TCP is approximately double that of (-)-TCP on the basis of the results obtained. It can be calculated that (-)-TCP would be 99% cleared from the brain within 318 min (7 half-lives) while (+)-TCP would require 609 min to decrease to the same level. Thus the biphasic elimination kinetics seen after chronic administration are not apparent after single doses.

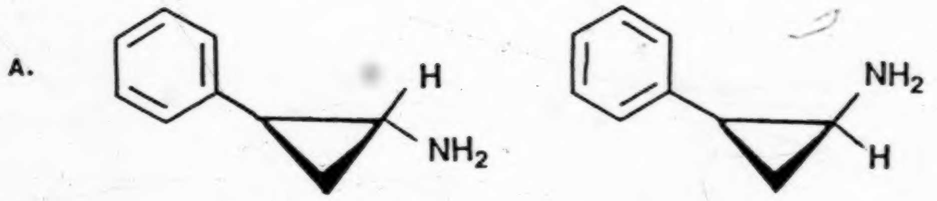
The present results are in partial agreement with those of Lang _et al._ (1979) who studied the disposition of TCP isomers in 10 volunteers given 20 mg of (+)- or (-)-TCP. Levels of isomers of TCP were monitored in plasma over 8 hr and in 24 hr urine collections. In apparent agreement with the results obtained in the present study on brain, Lang _et al._ (1979) observed that (-)-TCP enters the blood circulation more rapidly and attains a higher peak plasma concentration than the (+)-isomer. However, they reported that (-)-TCP appears to be metabolized and eliminated more slowly than (+)-TCP, a finding not in agreement with the present study. They also state, "in the urine collected over a period of 24 hr, the excretion rate of unmetabolized (+)-TCP was lower, probably resulting from the greater metabolization rate of this isomer." The phrase "probably resulting from the greater metabolization rate of this isomer" is speculative, since neither they nor others have provided any evidence that there is a difference in metabolism between the two isomers. However, the results from their urine data could be interpreted as being in agreement with the results obtained in the present study, in that (+)-TCP appears to be cleared from the brain more slowly than the (-) isomer and would therefore most likely be excreted in the urine more slowly. More extensive metabolic and pharmacokinetic studies and correlations between brain, blood, and urine are required before more definitive statements can be made.

The differential MAO-inhibiting properties of TCP isomers were most obvious in the in vitro experiments where it was found that the IC_{50} value for the (+)-isomer (0.16 µM) was almost 20x less than that for the (-)-isomer (3.00 µM) in the case of MAO-A, and almost 30x less in the case of MAO-B (0.10 µM vs. 2.77 µM). These results are in very good agreement with those of Fuentes et al. (1976) who found that between 10-60x higher concentrations of (-)-TCP were required to block enzyme activity by 50%, depending upon the amine substrate used. However, unlike the present results, Fuentes et al. (1976) found that both isomers inhibited MAO-B significantly greater than MAO-A ($p < 0.05$). Although the IC_{50} values for both isomers were lower for MAO-B than for MAO-A in the present study, the difference was not statistically significant ($p > 0.05$).

In light of these drastic changes induced by small changes in chemical structure, it is interesting to compare the absolute configurations of the TCP isomers to other MAO-inhibiting drugs. Riley and Brier (1972) have deduced that the absolute configuration of (+)-TCP is 1S:2R. This corresponds to the absolute configurations (S) of the reversible MAOI (+)-AMP (Granna and Lilla, 1959) and the irreversible MAOI pheniprazine (Bernstein et al., 1959). A structure-activity relationship (SAR) study (Zirkle et al., 1962) has demonstrated that the major binding moieties of this class of MAOIs are the phenyl and amino groups. It was also concluded that the inhibitory activity of arylcyclopropyl compounds is related to the ability of the phenyl group to approach coplanarity with the C-2, C-3 atoms of the cyclopropane ring. Neville _et al._ (1971) have reported that the conformational analysis of AMP as deduced by NMR indicates a preferred anti-conformation of the phenyl and amino groups. The anti-conformation, in which the amino group is above the plane of the benzene ring and with the side chain fully extended, corresponds to the trans-conformation of TCP (Horn and Snyder, 1972). Figure 29 illustrates the spatial relationships between AMP and TCP isomers more clearly. Finally, Miller and Clarke (1978) have shown that (+)-AMP is 4-fold more potent in inhibiting MAO-A than is (-)-AMP (no difference was observed between the two isomers in ability to inhibit MAO-B).

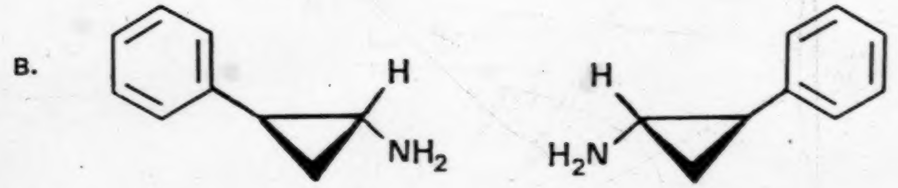
Thus (+)-TCP and (+)-AMP have been shown to possess the same absolute configuration and conformation in addition to displaying stronger MAO-inhibiting potencies over their respective (-)-isomers. It is probable that pheniprazine also exists in the anti-configuration and hence would also fit this stereochemical pattern.

The structural and stereochemical correlations found to exist for the MAO-inhibitory activity of TCP, AMP, and pheniprazine suggest very specific 3-dimensional structures are required for MAOIs. The resemblance of the inhibitor(s) to the substrate(s) is obvious with the older MAOIs, but this feature is less obvious with some of the newer reversible MAOIs (see Figure 1). In addition, the rigidity of the cyclopropyl ring of TCP undoubtedly enhances the drug-receptor interaction and consequently the MAO-inhibiting ability of both TCP isomers. Stereochemi-



trans-2-phenyl-cyclopropylamine

cis-2-phenyl-cyclopropylamine



(+)-TCP

(-)-TCP

Figure 29. The stereochemical relationship between A. the cis-trans isomers of 2-phenylcyclopropylamine and B. the optical isomers of tranylcypromine. In all four structures depicted, the CH_2 group of the cyclopropyl ring extends out of the plane of the paper towards the reader.

cal considerations of TCP isomers will be discussed further in Section IV.B.3.

2. Response of Biogenic Amines to Tranylcypromine Isomers

The differential response of the MAO-B substrate PEA to TCP isomers can be explained on the basis of the greater MAO-inhibiting properties of the (+)-isomer. The (+)-isomer dramatically elevated PEA levels at all time intervals after administration. In contrast, the (-)-isomer did not increase PEA at any time interval and in fact significantly decreased PEA levels at the 6.0 hr time period. The reasons for this decrease in PEA concentrations are unclear at this time. Intraperitoneal injection of (±)-TCP at a dose of 20 mg/kg (McKim _et al._, 1980) and intraventricular injection of (-)-TCP (Reigle _et al._, 1980) have been reported to cause a decrease in brain levels of NE under acute conditions; these decreases were thought to be due to the releasing effects of TCP on NE. It is conceivable that a similar effect could be occurring with PEA, but our knowledge about the storage of PEA is too inadequate at this time to permit us to make such a statement with confidence. Karoum _et al._ (1979) reported a bimodal distribution of PEA in rat brain, i.e. some rats had brain levels of this amine of about 10 ng/g, while others had levels which were much lower. A similar situation has been found in the present study, and it may be that all or most of the members of the group of rats under consideration had control values of PEA in the low range. Thus the variability encountered in the levels of endogenous PEA amplifies the difficulties involved in the interpretation of findings of decreased PEA levels with (-)-TCP.

The TCP isomers produced quite different effects on brain 5-HT levels compared to their effects on PEA. First, the (+)-isomer significantly increased 5-HT concentrations above control values at all time intervals, as was observed with PEA. However, unlike the response of PEA, brain 5-HT levels continued to increase throughout the first 6 hr after administration, although at each successive time interval the 5-HT concentration was not significantly greater than that at the interval immediately preceding. The opposite trend was observed with PEA, where the highest concentration was apparent 0.5 hr after injection and then decreased during the ensuing 5.5 hr. Again, only a trend was observed since none of these groups (time intervals) were significantly different from each other. The reasons for the slower and less dramatic response of 5-HT compared to PEA may be due to (a) the slower turnover rate of 5-HT relative to PEA (Durden and Philips, 1980) and (b) the slightly lower level of inhibition of MAO-A compared to MAO-B apparent at most time intervals in the _in vivo_ MAO experiments. The reasons for the trend towards decreasing PEA concentrations are not as obvious. Several hours is not enough time for synthesis of new enzyme since it has been shown that the turnover of MAO is approximately 12 days (Gordis and Neff, 1971). However, Maitre _et al._ (1976) have proposed that there may be two pools of MAO. In addition to the mitochondrial MAO they term "bulk MAO," they claim to have evidence for a small pool of MAO characterized by a rapid turnover (12-18 hrs) and relative resistance to inhibition. Evidence to support their claim is the observation that when radiolabelled MAO substrate precursors (e.g. L-dopa) are injected into rats pretreated with MAOIs, they accumulate in the brain in much greater amounts than in rats not pretreated with MAOIs. This effect, however, is of considerably shorter duration (half-life 12-18 hrs, depending upon the inhibitor used) than MAO inhibition (half-life 2.5-12 days). The observation of the present study, that there may be a trend towards a return to baseline levels of PEA within several hours after injection of (+)-TCP, tends to support the hypothesis of Maitre et al. (1976) that there is a small pool of MAO with a rapid turnover. In addition, one could speculate that this pool may be composed primarily of MAO-B since PEA, but not 5-HT, levels show a trend towards control values within 6 hr and that this trend also reflects the greater turnover rate of PEA versus 5-HT.

Rather interesting results were obtained with (-)-TCP. At 0.5 hr, 5-HT levels were not elevated above controls, but by 1.5 hrs they were significantly elevated. This corresponds to a low level of MAO-A inhibition (23.0%) at 0.5 hr with an increase to 43.8% by 1.5 hr. This level of inhibition remains approximately constant up to 6 hr. The 5-HT concentrations were highest at the 6 hr interval (significantly elevated above controls). These results are contrary to those of Smith and Petersen (1982) who found no elevations of brain 5-HT levels after administration of 1.5, 10, 15 and 30 mg/kg of (-)-TCP, although these same doses of (+)-TCP all did produce significant elevations of brain 5-HT levels. However, the rats in their study were all pretreated with a high dose of reserpine (10 mg/kg) which very likely could have obscured more subtle (but possibly significant) increases in 5-HT induced by (-)-TCP.

Interestingly, results from the present study indicate that inhibition of brain MAO-A by 40-50% is sufficient to substantially increase 5-HT concentrations in the brain. Luine and Fischette (1982) have provided evidence that low levels of inhibition of MAO-A are behaviorally

?

effective when inhibition is localized to the hypothalamus. They studied inhibition of lordosis behavior in estrogen-primed female rats by intrahypothalamic implants of pargyline. Their results show that pargyline applied directly to the hypothalamus results in antagonism of behavior at much lower levels of MAO inhibition than does peripheral administration. Lordosis responding was antagonized when hypothalamic MAO activity was inhibited by 40%.

The response of 5-HT to decreases of MAO activity of 40-50% may be restricted to certain brain areas such as the hypothalamus, but it is more likely that this phenomenon is ubiquitous since there is no indication that there are differences in inhibition kinetics of MAO-A in different brain regions.

In light of this, it is possible that environmental factors (e.g., stress, diet, or hormonal factors) could influence MAO activity. A substantial body of evidence has accumulated that indicates that gonadal hormones, particularly estrogen, can influence MAO activity. Kueng _et al._ (1976) have shown that both MAO activity and 5-HT levels change during the estrous cycle, and that there may be a relationship between the two. Luine and Paden (1982) state that "the magnitude of the physiological changes in the 5-HT systems are much smaller than the those observed here. However, since gonadal hormone effects on the serotonergic system appear to involve both pre- and post-synaptic changes occurring together, changes in several parameters may act synergistically to affect behavior. In addition, evidence presented in the following paper indicates that much lower levels of MAO inhibition are behaviorally effective when inhibition is localized to the hypothalamus. In fact, the decline of MAO activity following central application of pargyline is within the range of physiological changes that occur during the estrous cycle."

In a subsequent report (Luine and Paden, 1982), it was demonstrated that a clear dose-response relationship exists between decreases in lordosis and increases in preoptic hypothalamic 5-HT levels. In addition, stereotaxic application of 5-HT receptor blockers (methysergide, cinanserin) or PCPA to the preoptic hypothalamic area or lesions of ascending 5-HT pathways all facilitate lordosis responding in estrogen-treated female rats (Zemlan _et_ _al._, 1973).

Luine and Paden (1982) also measured preoptic hypothalamic MAO activity and 5-HT levels after administration of a variety of MAO inhibitors. Most of their drug treatments inhibited MAO-A activity by 90% or greater and increased 5-HT significantly. However, 50 mg/kg of pargyline plus 60 mg/kg of harmaline (a reversible MAO-A inhibitor) inhibited MAO-A by approximately 30% and elevated 5-HT slightly but not significantly ($p < 0.01$) above control values. These data along with data obtained from the present study indicate that inhibition of MAO-A by 40-50% may be the critical level of inhibition required to influence brain 5-HT concentrations.

An important implication of these results is that 5-HT levels may be more responsive to low levels of MAO inhibition than was previously thought. It has been widely accepted that 80-90% inhibition is the minimum required for therapeutic response (Robinson _et_ _al._, 1978) and to affect amine levels (Green _et_ _al._, 1977). In addition, these results may help to corroborate findings by others of altered physiological and biochemical parameters seen in normal persons with low platelet MAO-B activity. For example, Kleinman _et_ _al._ (1979) have reported that a correlation exists between low platelet MAO activity and high plasma prolactin concentrations (DA is inhibitory towards prolactin release in the pituitary). It appears that there is a threshold beneath which platelet MAO activity is associated with plasma prolactin concentrations. In persons with high platelet MAO activity, no correlation was found. Mendelson _et al._ (1979) reported that persons with low platelet MAO activity had significantly more total REM sleep time and REM density that persons with high platelet MAO activity.

Thus it appears that low to moderate reductions in MAO-A or MAO-B activity, either produced pharmacologically or due to inherently low activity in normal people, may have important physiological consequences that are not yet fully understood.

3. Effects of Tranylcypromine Isomers on Inhibition of Reuptake and Release of Neurotransmitters

Both TCP isomers inhibited the reuptake of tritiated DA, NE, and 5-HT in neuronal prisms. With all 3 neurotransmitters tested, (-)-TCP was the more potent of the two isomers. The highest degree of selectivity was seen with DA. These results are in agreement with those of Horn and Snyder (1972) who also reported that the (-)-isomer was a stronger inhibitor of catecholamine reuptake in rat brain.

The simplest form of stereoselectivity is based on the so-called three point attachment to the receptor (Tuomisto, 1978). If the molecule binds to the receptor through 3 groups whose positions are not interchangeable, the enantiomer (mirror image isomer) of the molecule is not able to bind to the receptor in the same manner.

At least one other MAOI which contains a chiral center, and thus exists in 2 stereoisomeric forms, has been shown to possess differential MAO-inhibiting and amine reuptake-inhibiting abilities between the two isomers. (-)-Deprenyl is a potent and specific MAO-B inhibitor; (+)-deprenyl is less potent as an MAO inhibitor but has stronger amine reuptake-inhibiting properties than the (-)-isomer (Knoll, 1976). The absolute conformation of deprenyl has not been elucidated.

As stated previously (Section IV.B.1), (+)-TCP corresponds stereochemically to (+)-AMP and both of these compounds are more potent inhibitors of MAO than their corresponding (-)-isomers. However, unlike (-)-TCP, which is the stronger of the two TCP isomers in inhibiting reuptake and stimulating release of amines, (+)-AMP is also the stronger AMP isomer in inhibiting reuptake and facilitating release of amines (Horn and Snyder, 1972).

Tranylcypromine is particularly well suited to studies on neurotransmitter dynamics, including elucidation of amine uptake sites. Conventional SAR studies on phenylalkylamines or indolealkylamines are only of limited value for conformational analysis, since these compounds have flexible side-chains capable of assuming an infinite number of positions. Although the conformational analysis of these amines by various mathematical methods may reveal that certain conformations are more favorable than others, the energy differences between the conformations are very small, e.g. 1-2 Kcal/mole (Grol and Rollema, 1977). Since freely rotating molecules cannot be used to reveal the conformation necessary for binding to the receptor, various rigid derivatives, such as the TCP isomers, have been used to give more definitive information as to the characteristics of amine uptake sites. Other rigid molecules used to study amine uptake include 2-aminoindanes (Horn and Snyder, 1972), trans-decalin derivatives (Tuomisto et al., 1976), and 2-aminobenzobicyclo(2,2,2)octene isomers (Grunewald et al., 1976). All these compounds are rigid analogs of AMP.

A major drawback of these SAR studies is that incorporation of additional groups into the basic phenylethylamine or AMP structure renders them bulkier and, in many cases, changes the lipophilicity dramatically. Also, many of these analogs, due to the presence of additional chiral centers, are diastereomers, and hence their physicochemical properties may differ substantially from their endogenous substrates. This makes it difficult to determine whether it is the change in conformation or the change in the physicochemical characteristics which causes the altered pharmacological effect.

Despite the limitations of some of these studies, the following general conclusions can be drawn with regard to biogenic amine uptake sites: (1) Some basic features are obligatory for a molecule to bind to the uptake site. These include a protonated N atom separated by 1 or 2 carbon units from a phenyl ring. An additional ancillary group alpha to the N atom, such as the methyl group of AMP or the various structures of the rigid analogs, can greatly increase the capacity for inhibition of uptake and/or releasing of biogenic amines. (2) Some stereochemical features can profoundly affect the pharmacological activity of a molecule, while others appear to have minimal influence. For example, the work of Horn and Snyder (1972) has shown that the conformation of TCP isomers has a dramatic effect on amine uptake inhibition. The cis isomer of TCP (cis-2-phenylcyclopropylamine) is 600x less potent than the trans isomer (TCP) in inhibiting NE reuptake in the hypothalamus. One could assume that the trans or anti conformation (inherent in both optical isomers of TCP) is one of the most important stereochemical features of inhibitors of reuptake. The trans conformation has also been demonstrated to be the preferred conformation for the amine substrates themselves (NE [Carlstrom and Bergin, 1967], DA [Bergin and Carlstrom, 1968]). The relative lack of effect of configuration is understandable, at least in the cases of DA and 5-HT, since neither of these neurotransmitters exists in isomeric form itself. Finally, (3) in general, the postsynaptic sites for DA, NE, and 5-HT appear to be much more strict in terms of structural deviations than are the uptake sites. Although a wide variety of compounds with diverse molecular structures, including the tricyclic antidepressants, some of the newer "second and third generation" antidepressants, and TCP isomers, are all capable of inhibiting NE and/or 5-HT reuptake, some drugs, such as nomifensine and benztropine, are relatively specific DA reuptake inhibitors. Eckhardt et al. (1982) has demonstrated that there is a difference between the NE uptake sites in the hypothalamus and those of the DA uptake sites in the striatum. Their data, obtained by studying uptake inhibition by a variety of substituted substrate molecules, have indicated that there is a great similarity, if not identity, in the receptive area for substrates in striatum (DA) and hypothalamus (NE). Although the substrates and inhibitors bind to some groups in common in the substrate receptive area, it is the surrounding hydrophobic molecular environment that is clearly different and permits the phenomenon of selective blockage with some drugs.

In summary, it has been shown that although both (+)- and (-)-TCP have the capability of inhibiting reuptake of biogenic amines into neuronal prisms, the (-)-isomer is the more potent of the two in both respects in the case of DA and 5-HT. The concentrations of TCP in the brain (approximately 5×10^{-6} M) after chronic administration of a clinically relevant dose (1 mg/kg) are capable of substantially inhibiting the reuptake of NE, DA, and 5-HT into neurons. The amine releasing abilities of TCP are not apparent at these concentrations and probably contribute relatively little to the overall pharmacological effects when compared to the MAO-inhibiting and reuptake-inhibiting properties of the drug. However, many experiments in laboratory animals reported in the literature utilize doses of TCP ranging from 5 to 20 mg/kg. At the concentrations attained in brain after these doses (brain levels of TCP in excess of 10^{-4} M were reported by Calverley *et al.* [1981] 1.5 h after administration of 20 mg/kg of TCP HCl), TCP can have marked effects on uptake *and* release of the catecholamines and 5-HT (as shown in the *in vitro* experiments reported in this thesis), and these actions may well contribute to the overall pharmacological profile of the drug under these circumstances. Thus it appears futile to debate whether it is the inhibition of amine reuptake or the MAO-inhibiting properties of TCP that are responsible for its therapeutic efficacy since TCP exhibits both properties, and other drugs which are "pure" MAOIs or "pure" reuptake inhibitors are known to be effective antidepressants.

IV.C. The Detection and Characterization of Para-Hydroxytranylcypromine

1. Detection of Para-Hydroxytranylcypromine

Three analytical procedures were attempted in an effort to quantitate *p*-OH-TCP in brain:

(1) Acetylation followed by pentafluorobenzoylation as utilized for analysis of TCP (Hampson *et al.*, 1984a). This resulted in a deri-

vative of p-OH-TCP with poor GC peak shape and low sensitivity.

(2) Pentafluorobenzoylation in basic aqueous medium (see Section II.F.2 in Methods for further details). Although a suitable derivative was formed, a large interfering peak was present in brain tissue extracts. An interfering peak was also present in urine, but levels of p-OH-TCP were considerably higher than the levels of this interfering substance and therefore control (non-drug-treated) urines were run in parallel and the small peak produced by the interfering substance was subtracted from the total peak height.

(3) The method described by Baker et al. (1983) was utilized for analysis of p-tyramine in brain (see Section II.F.2(b) in Materials and Methods for details). This involves acetylation, specific hydrolysis of acetylated phenols, and reaction with trifluoroacetic anhydride to produce N-acetyl,N,O-di(trifluoroacetyl)-p-OH-TCP. Unfortunately, the peak height of the derivatized p-OH-TCP in brains from rats treated with 1 mg/kg TCP was not significantly greater than that of the baseline noise in control brains, and an adequate quantitation could not be performed in the drug-treated animals. It thus appears that other methods, such as HPLC with electrochemical detection, may have to be utilized to study levels of p-OH-TCP in brains of rats treated with a low dose of TCP. However, when brains from rats treated with 10 mg/kg TCP were carried through the same procedure, a sharp peak approximately 10 times baseline noise levels and co-chromatographing (on two different GC columns) with derivatized p-OH-TCP standard was observed. This peak was shown by CI-SIM-GC-MS to be derivatized p-OH-TCP.

Further supportive evidence for the presence of p-OH-TCP in brain was provided by indirect, pharmacological experiments in which TCP levels in brain were observed with and without prior pretreatment with drugs known to block ring hydroxylation. All the drugs tested caused significant increases in brain concentrations of TCP, indicating that ring hydroxylation may be of some importance in the metabolism of TCP. The rather strong effect of several antipsychotic drugs on increasing brain levels of TCP is of interest, since such drugs are sometimes given in conjunction with TCP in clinical situations (see Section I.C.7 of Introduction). Antipsychotic drugs are known to interfere with hydroxylation of tricyclic antidepressants when these drugs are co-administered, and this effect is thought to be due to competition for the hepatic cytochrome P-450 system that metabolizes both compounds (Siris et al., 1982). This is apparently the first time that a similar interaction with TCP has been reported.

It is too early at this time to predict if the p-OH-TCP detected in the brain is formed there entirely or if there is a contribution from the liver and/or other peripheral sources. A study by Coutts et al. (1984) on p-OH-AMP strongly suggests that the amounts of this compound present in rat brain after intraperitoneal injection of AMP are due to formation within the brain rather than passage into the brain from peripheral sources.

It is conceivable that the p-OH-TCP present in brain could be taken up by nerve terminals, since Cho et al. (1977) demonstrated that p-OH-AMP is accumulated by synaptosome-enriched preparations prepared from both cortical and striatal tissue.

The unequivocal presence of p-OH-TCP in urine from rats dosed with TCP was also demonstrated by GC and SIM-MS. The fact that p-OH-TCP was detected in urine after a 1 mg/kg dose of TCP indicates that p-OH-TCP may have some clinical relevance since this dose is comparable to that used in a therapeutic situation. In addition, the biological activity of p-OH-TCP, which has also been demonstrated (see the following section), is further evidence of a potential role for this metabolite in the overall effectiveness of TCP as an antidepressant. It should also be mentioned that only free p-OH-TCP was measured in the urine, since initial attempts at acid hydrolysis apparently resulted in degradation of p-OH-TCP. Coutts et al. (1976) have shown that the majority of p-OH-AMP detected in rat urine is present in the conjugated form. Thus it is quite possible that the free urinary p-OH-TCP detected in the present study represents only a fraction of the total metabolite formed. Further studies utilizing milder procedures to free conjugated p-OH-TCP thus appear to be warranted.

The reduction of p-OH-TCP levels in the urine to below the limits of detectability in iprindole-pretreated rats is further proof that iprindole inhibits para-hydroxylation. Freeman and Sulser (1972) also reported that p-OH-AMP was "reduced to negligible amounts" in the urine of iprindole-pretreated rats dosed with AMP.

In a previous report, Alleva (1965) studied the metabolism of C^{14}-TCP and C^{14}-AMP in the rat. Urine, feces, and expired CO_2 were examined for radioactivity, and it was determined that the 24-hr urine collection contained about 75-80% of the total injected radioactivity for both drugs. Approximately 10% of the total radioactivity was recovered in the feces and only traces of C^{14}-CO_2 were recovered in expired air of C^{14}-TCP-treated rats. Of the 6 C^{14} peaks found in paper chromatograms of urine samples from C^{14}-TCP-treated rats, only 3 were identified: C^{14}-

hippuric acid, the major metabolite, which accounts for 80% of the total urine radioactivity; unchanged TCP, which accounts for 6% of the total urine radioactivity; and benzoic acid (4% of urine radioactivity). None of the other peaks could be identified, although it was confirmed that none was AMP, since all metabolites had R_f values which differed from that of AMP. It is possible that one of these peaks may have been p-OH-TCP, or N-acetyl-TCP, another known metabolite of TCP (Calverley et al., 1981), although no mention was made of these metabolites in this report. Their inability to detect AMP as a TCP metabolite in urine was confirmed in the present study. All attempts to detect AMP as a TCP metabolite in rat brain were unsuccessful.

Youdim et al. (1979) have published one of the few, if not the only, report on the detection of AMP as a TCP metabolite using a GC-MS procedure. They also claim to have detected methamphetamine as a metabolite of TCP, although they admit that there was an interfering substance which comigrated with this peak. Also, it must be kept in mind that this report was the result of a study on a single patient who had overdosed on 250 mg of TCP, and therefore this case is not germane to the present study or to a therapeutic situation.

Since the present study has demonstrated the presence of p-OH-TCP, it may be hypothesized that this metabolite could be further hydroxylated to form 3,4-dihydroxytranylcypromine. Hoffman et al. (1979) have described the formation of a catechol metabolite of AMP, α-methyldopamine, by rat brain microsomes. The enzyme involved in the hydroxylation of p-OH-AMP was described as a cytochrome P-450-dependent monooxygenase. However, since the Km of the catechol-forming enzyme probably exceeds the concentration of p-OH-AMP, it is unlikely that substantial amounts of α-methyldopamine are normally synthesized. In the case of TCP, however, where chronic treatment and accumulation in the brain has been demonstrated, it is possible that some proportion of p-OH-TCP is further metabolized to 3,4-dihyroxytranylcypromine. The structure of this metabolite is similar to the endogenous catecholamines and therefore it may display some degree of activity at postsynaptic catecholamine receptors.

The possibility of the catechol metabolite being methylated by catechol-O-methyl transferase (COMT) to form 4-OH-3-methoxy-TCP also exists. Coutts *et al.* (1976) have described both p-OH-AMP and 4-OH-3-methoxy-AMP as *in vivo* metabolites of AMP in the rat. They reported that these urinary metabolites accounted for 18.3% and 3.3% respectively of the administered dose.

2. Characterization of the Biological Activity of Para-Hydroxytranylcypromine

The biological activity of p-OH-TCP was tested in three ways: for inhibition of MAO (*in vitro*), ability to inhibit the reuptake of tritiated neurotransmitters into neuronal prisms, and the capacity to release tritiated neurotransmitters from neuronal prisms.

Para-hydroxytranylcypromine was a potent inhibitor of both MAO-A and MAO-B. In fact, p-OH-TCP was essentially equipotent to TCP itself in inhibiting both forms of the enzyme. *Para*-hydroxytranylcypromine was also approximately equipotent to TCP in inhibiting the reuptake of ^{3}H-DA and ^{3}H-NE and in causing release of these tritiated catecholamines in striatal and hypothalamic tissue respectively. Surprisingly, the hydroxylated compound was considerably more potent than TCP in inhibiting the reuptake of ^{3}H-5-HT and in promoting the release of ^{3}H-5-HT from striatal tissue. Thus, p-OH-TCP appears to retain the full activity of the parent drug, except for its effects on ^{3}H-5-HT (inhibition of reuptake and releasing ability), in which it is actually more potent than TCP itself.

Although p-OH-TCP has as great, or greater, potency than the parent drug with regard to the above actions, a number of differences may be important with regard to para-hydroxylation. Again, the use of AMP for comparative purposes may be advantageous, since more research in this area has been conducted on AMP than on TCP. Taylor and Sulser (1973) have studied the effects of AMP and p-OH-AMP on central noradrenergic systems. They found that when these amines were administered intraventricularly, p-OH-AMP had a half-life of 2.5-5.2 times as long as AMP (half-life, 15 min). Also AMP administration resulted in predominantly stereotyped behavior while p-OH-AMP caused predominantly locomotor stimulation. Although both AMP and p-OH-AMP blocked the reuptake of ^{3}H-NE, only p-OH-AMP significantly reduced ^{3}H-NE levels and increased the levels of the O-methylated metabolite, normetanephrine. They concluded that p-OH-AMP had stronger releasing properties than AMP, and that both this effect and the differential behavioral responses may be attributed to the much longer half-life of the metabolite.

Another important factor that is germane to this discussion is the effect of para-hydroxylation on the uptake of the drug itself. In a study by Lentzen and Philippu (1981) on the uptake dynamics of NE, DA, p-TA, AMP, octopamine, and PEA, it was also observed that para-hydroxylation enhanced uptake. They concluded that the hydroxylated compounds were accumulated into vesicles by an ATP-Mg^{2+}-dependent process, while non-hydroxylated PEA analogs (PEA, AMP) were taken up by ATP-Mg^{2+}-independent process, i.e. diffusion.

In summary, the results obtained in the present study have shown that p-OH-TCP is an active metabolite of TCP. Its ability to inhibit the reuptake of ^{3}H-5-HT and release ^{3}H-5-HT from striatal tissue exceeds that of TCP itself. It is also apparent from the results of other studies that p-OH-TCP may be transported across neuronal membranes and taken up into vesicles in a manner that is quite different from TCP.

1. Detection of 6-Hydroxytetrahydro-β-Carboline

Initial attempts to replicate the work of Barker et al. (1981), who reported 500 ng/g of THBC and 1114 ng/g of 6-methoxy-THBC in adrenal gland of control rats, were unsuccessful. Attempts to detect these compounds plus 6-OH-THBC in brain were also unsuccessful. Although in the present study, GC with glass capillary columns and electron-capture detection was utilized rather than MS as used by Barker et al. (1981), it was felt that the problems encountered in detection were not due to a lack of sensitivity with GC, but to technical difficulties in the method (e.g. loss of sample due to extensive work-up procedures). It was therefore decided that development of a better method would be advantageous. It was also felt that work should be concentrated on the detection of 6-OH-THBC since this was the THBC most likely to be present in the highest quantity and because its detection in the rat has never been reported.

The double-derivatization procedure developed entailed a phase-transfer reaction with PFBZ to extract 6-OH-THBC from aqueous media (urine) followed by reaction with PFPA. The second derivatization was necessary because reaction of 6-OH-THBC with PFBZ alone consistently produced a derivative of low volatility, poor peak shape, and low sensitivity. It was assumed that reaction of 6-OH-THBC with PFBZ resulted in an O-PFBZ, N-2-PFBZ derivative. This assumption was based on the observation that THBC reacted with PFBZ to form the N-2-PFBZ derivative (as confirmed by MS). The N-2-PFBZ moiety of the O-PFBZ, N-2-PFBZ-6-OH-THBC derivative was apparently replaced by a PFP group after reaction with PFPA to form the derivative whose structure was confirmed by MS (Figure 27). Although the molecular weight did not differ substantially from the PFBZ derivative, the replacement of one pentafluorobenzoyl group with a pentafluoropropionyl group apparently changed the volatility enough to produce a derivative which chromatographed adequately.

Utilizing the method developed (Section II.F.3), 6-OH-THBC was detected in the urine of rats treated with TCP and 5-hydroxytryptophan. A portion of 6-OH-THBC appeared to be conjugated since in urine samples which were not treated with glusalase, levels of 6-OH-THBC were reduced compared to glusalase-treated urines, in some cases to levels below the limit of detection of the assay (approximately 100 ng/ml). 6-Hydroxytetrahydro-β-carboline could not be detected in glusalase-treated urines from control rats. These results are in agreement with those of Rommelspacher _et al._ (1979) who reported the detection of 6-OH-THBC in human urine after a 300 mg dose of 5-hydroxytryptophan. These workers were also unable to detect 6-OH-THBC in the urine of control subjects. In addition, they reported that 6-OH-THBC was conjugated with glucuronic acid in humans. Beck and Lundman (1983) reported the detection of 6-OH-1-methyl-THBC in urine from untreated rats and that 90% of the compound appeared in conjugated form. They also detected this THBC in liver.

6-OH-1-methyl-tetrahydro-β-carboline is the condensation product of 5-HT with the ethanol metabolite acetylaldehyde. None of these studies, including the present study, was able to detect THBCs in rat brain tissue. In the study of Rommelspacher et al. (1979), a possible explanation for the failure to detect 6-OH-THBC in brain would be substantial sample loss during the extensive workup and purification procedures, but this does not explain similar results in the present study, since this procedure entailed relatively little sample manipulation.

Although sensitivity with the method developed in this study was good, reproducibility and linearity were not. Other derivatizing agents (e.g. heptafluorobutyric anhydride and heptafluorobutyrylimidazole) were tried, but similar problems were encountered with these agents. Numerous attempts to determine the cause of these problems were not successful. Therefore, it was felt that quantitation of 6-OH-THBC with this method could not be carried out. Subsequently, an attempt was made to develop a method for analysis of 6-OH-THBC using HPLC with fluorescence detection. Several THBCs, including 6-OH-THBC, and several of their oxidized analogs (β-carbolines) were chromatographed on a Waters HPLC equipped with a C_{18}-reverse phase column and a fluorescence detector (excitation, 264 nm; emission, 390 nm). Although the fully oxidized β-carbolines were very sensitive to fluorescence detection, their reduced congeners (THBCs) proved to be much less sensitive. Therefore, it was decided that this method of analysis would not be suitable for use in the present study due to the low sensitivity of THBCs to fluorescence detection.

Despite the problems involved in quantitation, the results of urine analysis by SIM-MS (Figure 28) reveal that 6-OH-THBC is found in rat urine after dosing with TCP and 5-hydroxytryptophan. This implies that the rat may indeed be a suitable animal model for further studies on THBC and β-carboline formation in vivo. Rommelspacher et al. (1979) have demonstrated that 6-OH-THBC does possess biological activity, including induction of hypothermia, catalepsy and nociception. In the experiments of these workers, 6-OH-THBC was injected intracisternally because it was also demonstrated that this compound did not cross the blood-brain barrier. Despite this observation and the failure to detect 6-OH-THBC in brain tissue in this study and the present study, it is conceivable that 6-OH-THBC is formed in small amounts in the brain (i.e. its levels are below the limit of detection). It would be advisable in future studies to concentrate on brain substructures which contain higher levels of 5-HT, for example, the hypothalamus and the raphe nuclei, to increase the probability of detection of 6-OH-THBC. Another possible explanation for the inability to detect 6-OH-THBC in the CNS may be due to its oxidation to 6-OH-β-carboline. The oxidation of THBCs to their corresponding β-carbolines has been reported (Greiner and Rommelspacher, 1982). If this were the case, the N-2 atom would become a tertiary N and would therefore not react readily with most derivatizing reagents including PFBZ or PFPA. As alluded to above, the β-carbolines formed from THBCs by oxidation at N-2 position may be more amenable to analysis by HPLC with fluorescence detection.

CONCLUSIONS AND RECOMMENDATIONS

V. CONCLUSIONS AND RECOMMENDATIONS

Based upon the findings of this study, the following general conclusions can be made:

(1) A single 1 mg/kg dose of TCP is sufficient to cause approximately 87% inhibition of MAO-A and MAO-B in the rat CNS. This level of enzyme inhibition is responsible for the immediate elevation of brain 5-HT, DA, PEA, and T. Urinary excretion of 5-HT, T, PEA, and 3-MT (a metabolite of DA) also increased immediately.

(2) Peak levels of T, PEA, and DA appear to be reached after the first few days of dosing, and these levels are maintained for up to 6 weeks of drug administration. Brain 5-HT concentrations increase steadily up to 21 days, after which a plateau is reached. Levels of TCP followed a similar pattern. Brain 5-HT levels correlated highly with the brain TCP concentrations. Both of these parameters coincided temporally with the typical time required for the onset of therapeutic effect in clinical situations.

Although other antidepressant drugs may show different effects on biogenic amine levels after chronic treatment, it is apparent from this study that highly elevated amine levels are maintained in the CNS during chronic TCP administration. It is likely that other nonspecific MAOIs produce similar effects. It is also probable that these long-term elevations in CNS amine concentrations may be responsible for the down-regulation of postsynaptic 5-HT receptors and adrenoceptors that is associated with most (if not all) antidepressant treatments. More research on this aspect of TCP and other MAOIs would be advantageous since both pre- and post-synaptic receptors function together in a holistic manner.

(3) The optical isomers of TCP produce different neurochemical effects in the CNS. The (+)-isomer is a much more potent inhibitor of both forms of MAO, while the (-)-isomer is a more potent inhibitor of amine (DA, NE, 5-HT) reuptake and a stronger releaser of DA and 5-HT than is the (+)-isomer.

The potent and multifaceted neurochemical properties of TCP are most likely responsible for the high response rate and rapid onset of action seen with this drug. Some properties may actually be synergistic, e.g. amine-releasing and reuptake-inhibiting properties.

(4) Acetylation under aqueous conditions followed by pentafluorobenzoylation under anhydrous conditions and quantitation by electron-capture gas chromatography with high-resolution capillary columns has proven to be a rapid, sensitive procedure for routine analysis of both TCP and PEA in brain tissue and urine. This method also has potential for analysis of other bioactive amines (e.g. amphetamine) in tissues and body fluids.

(5) Tranylcypromine is metabolized to *p*-OH-TCP in the rat. This hydroxylated metabolite has been identified in rat brain and urine. The detection and quantitative analysis of *p*-OH-TCP in urines from patients treated with TCP would substantiate the clinical relevance of this metabolite.

(6) Tests for biological activity of *p*-OH-TCP have revealed it to be an active compound. Its potency in inhibiting both forms of MAO is equivalent to that of TCP itself. This metabolite is also equipotent to TCP in inhibiting the reuptake and in promoting the release of catecholamines from striatal and hypothalamic tissue. *Para*-hydroxytranylcypromine is significantly more potent than the parent drug in causing

release and inhibiting the reuptake of 5-HT from neurons. Based on these observations, the possibility exists that a changing neurochemical profile may occur during chronic TCP administration, depending on the relative amounts of TCP/p-OH-TCP. Additional studies which attempt to determine the relative importance of TCP/p-OH-TCP, especially after long-term drug administration, are certainly warranted. The determination of the extent to which para-hydroxylation occurs in the brain itself would also be valuable. Finally, the extent of conjugation of p-OH-TCP would provide additional useful information on the biodegradation of TCP.

(7) Amphetamine does not appear to be a metabolite of TCP in the rat CNS, at least at a 1 mg/kg dose. In an overdose situation, the formation of AMP from TCP may contribute to the central stimulation seen with TCP at extremely high doses.

(8) The formation of the condensation product of 5-HT with formaldehyde, 6-OH-THBC, has been demonstrated in the rat after dosing with TCP and 5-hydroxytryptophan. The demonstration of 6-OH-THBC in vivo provides grounds for postulating the existence of other compounds of a similar nature. The rat appears to be a suitable animal model for use in such studies. The existence of other β-carbolines has been demonstrated by others under different conditions, i.e. different drug treatments. The effects of the diet (e.g. alcohol or foods with a high tryptophan content) on the formation of these compounds would also provide useful information. Further characterization of the neurochemical properties of β-carbolines and THBC congeners may give insights into the in vivo function of these compounds. The possibility of a β-carboline-related compound functioning as an endogenous ligand or modulator of the BZD

receptor also warrants further investigation.

(9) The analytical problems involved with the analysis of 6-OH-THBC and related β-carboline congeners were formidable. The large molecular size and weight, insolubility, and amphoteric nature were detrimental to the quantitative analysis of this compound. The problems associated with the high molecular weight of 6-OH-THBC extended to its volatility in GC analysis. The problems of both volatility and sensitivity preclude the use of other GC detectors such as flame-ionization detectors which do not require the use of ECD-sensitive derivatives. Other analytical methods such as radioimmumoassay or HPLC with electrochemical detection (for use with THBCs and phenolic β-carbolines) or fluorescence detection (for β-carbolines) may be more conducive to accurate and reproducible quantitation of this class of compounds.

BIBLIOGRAPHY

Aghajanian, G. K. (1972). Influence of drugs on the firing of serotonin-containing neurons in brain. Fed. Proc. Am. Soc. Exper. Biol., 31: 91-96.

Airaksinen, M. M. and Kari, I. (1981). β -Carbolines, psychoactive compounds in the mammalian body. Med. Biol., 59: 21-34.

Alleva, J. J. (1965). Metabolism of tranylcypromine-C^{14} and dl-amphetamine-C^{14} in the rat. J. Med. Chem., 6: 621-624.

Axelrod, J. (1954). Studies on sympathomimetic amines II. The biotransformation and physiological disposition of D-amphetamine and D-p-hydroxyamphetamine. J. Pharmacol. Exp. Ther., 110: 315-326.

Axelrod, J. (1955). The enzymatic deamination of amphetamine (benzedrine). J. Biol. Chem., 214: 753-763.

Baker, G. B. and Coutts, R. T. (1982). Amines of biological interest and their analysis. In: Analysis of Biogenic Amines (Baker, G. B. and Coutts, R. T., eds), pp. 1-14. Elsevier, Amsterdam.

Baker, G. B., Coutts, R. T. and Martin, I. L. (1981). Measurement of amines in the central nervous system using gas chromatography with electron-capture detection. Progr. Neurobiol., 17: 1-24.

Baker, G. B., Hiob, L. A. and Dewhurst, W. G. (1980). Effects of monoamine oxidase inhibitors on release of dopamine and 5-hydroxytryptamine from rat striatum in vitro. Cell. Mol. Biol., 26: 182-186.

Baker, G. B., LeGatt, D. F. and Coutts, R. T. (1982). A gas chromatographic procedure for quantification of para-tyramine in rat brain. J. Neurosci. Methods, 5: 181-188.

Baker, G. B., Martin, I. L., Coutts, R. T. and Benderly, A. (1980). Determination of 5-hydroxytryptamine in rat brain regions using gas chromatography with electron-capture detection. J. Pharmacol. Methods, 3: 173-179.

Bannon, M. J., Michaud, R. L. and Roth, R. H. (1981). Mesocortical dopamine neurons: Lack of autoreceptors modulating dopamine synthesis. Mol. Pharmacol., 19: 270-275.

Barker, S. A., Harrison R. E., Monti, J. A., Brown, G. B. and Christian, S. T. (1981). Identification and quantitation of 1,2,3,4-tetrahydro-β -carboline, 2-methyl-1,2,3,4-tetrahydro-β -carboline, and 6-methoxy-1,2,3,4-tetrahydro-β-carboline as in vivo constituents of rat brain and adrenal gland. Biochem. Pharmacol., 30: 9-17.

Beck, O., Bosin, T. R., Holmstedt, B. and Lundman, A. (1982). A GC-MS study on the occurrence of two tetrahydro-β-carbolines implicated in alcoholism. In: Beta-Carbolines and Tetrahydroisoquinolines (Bloom, F., Barchas, J. D., Sandler, M. and Usdin, E., eds.), pp. 29-40. Alan R. Liss, Inc., New York.

Belanger, P. M. and Atitse-Gbeassor, A. (1982). Inhibitory effect of tranylcypromine on hepatic drug metabolism in the rat. Biochem. Pharmacol., 31: 2679-2683.

Bergin, R. and Carlstrom, D. (1968). The structure of the catecholamines. II. The crystal structure of dopamine hydrochloride. Acta Crystallogr. Sect., B24: 1506-1510.

Bernstein, J., Losee, K. A., Smith, C. I., and Rubin, B. (1959). A novel resolution of 1-phenyl-2-propylhydrazine. J. Amer. Chem. Soc., 81: 443-445.

Bieck, P. R. and Antonin, K. H. (1982). Monoamine oxidase inhibition by tranylcypromine: Assessment in human volunteers. Eur. J. Clin. Pharmacol., 22: 301-308.

Bieck, P. R., Nilsson, E., Schick, C., Waldmeier, P. C. and Lauber, J. (1984). Urinary excretion of tryptamine in comparison to normetanephrine and beta-phenylethylamine in human volunteers after subchronic treatment with different monoamine oxidase inhibitors. In: Neurobiology of the Trace Amines. Analytical, Physiological and Pharmacological Aspects (Boulton, A. A., Baker, G. B., Dewhurst, W. G. and Sandler M., eds), pp. 525-542. Humana Press, Clifton, N.J.

Billings, R. E., Murphy, P. J., McMahon, R. E. and Ashmore J. (1978). Aromatic hydroxylation of amphetamine with rat liver microsomes, perfused liver, and isolated hepatocytes. Biochem. Pharmacol., 27: 2525-2529.

Bloom, F., Barchas, J., Sandler, M. and Usdin, E. (1982). Beta-Carbolines and Tetrahydroisoquinolines. Alan R. Liss, Inc., New York.

Bock, U. E. G. and Waser, P. G. (1981). Gas chromatographic determination of some biogenic amines as their pentafluorobenzoyl derivatives in the picogram range and its applicability to biological materials. J. Chromatography, 213: 413-428.

Bosin, T., Holmstedt, B., Lundman, A. and Beck O. (1982). Analytical pitfalls in the identification and measurement of endogenous tetrahydro-β-carbolines. In: Beta-Carbolines and Tetrahydroisoquinolines (Bloom, F., Barchas, J. D., Sandler, M. and Usdin, E., eds.), pp. 15-28. Alan R. Liss, Inc., New York.

Boulton, A. A. (1976). Cerebral aryl alkyl aminergic mechanisms. In: Trace Amines and the Brain (Usdin, E. and Sandler, M., eds), pp. 22-39. Marcel Dekker, New York.

Boulton, A. A. (1980). Trace amines and mental disorders. Can. J. Neurol. Sci., 7: 261-262.

Bourgoin, S., Artaud, F., Adrien, J., Hery, F., Glowinski, J. and Hamon, M. (1977). 5-Hydroxytryptamine catabolism in the rat brain during ontogenesis. J. Neurochem., 28: 417-422.

Bowers, M. B. and Salomonsson, L. A. (1982). LSD: Effect on monoamine metabolites in rat prefrontal cortex. Biochem. Pharmacol., 31: 4093-4096.

Braestrup, C., Nielsen, M., and Olsen, C. E. (1980). Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. Proc. Natl. Acad. Sci. U.S.A., 77: 2288- 2292.

Buckholtz, N. and Boggan, W. O. (1977). Inhibition by β-carbolines of monoamine uptake into a synaptosomal preparation. Life Sci., 20: 2093-2100.

Buckholtz, N. (1980). Neurobiology of tetrahydro-β-carbolines. Life Sci., 27: 893-903.

Calverley, D. G., Baker, G. B., Coutts, R. T., and Dewhurst, W. G. (1981). A method for measurement of tranylcypromine in rat brain regions using gas chromatography with electron-capture detection. Biochem. Pharmacol., 30: 861-867.

Campbell, I. C., Robinson, D. S., Lovenberg, W. and Murphy, D. L. (1979) The effects of chronic regimens of clorgyline and pargyline on monoamine metabolism in rat brain. J. Neurochem., 32: 49-55.

Carlstrom, D. and Bergin, R. (1967). The crystal structure of the catecholamines. I. The crystal structure of noradrenaline hydrochloride. Acta Crystallogr. Sect., B23: 313-319.

Cascio, C. S. and Kellar, K. J. (1983). In vivo regulation of ^{3}H-tryptamine binding sites in rat brain. 13th Annual Meeting of the Society for Neuroscience, Boston, Mass. (abstract #333.4).

Cascio, C. S. and Kellar, K. J. (1982). Tetrahydro-beta-carbolines: Affinities for tryptamine and serotonergic binding sites. Neuropharmacol., 21: 1219-1221.

Cho, A. K., Fischer, J. F., and Schaeffer, J. C. (1977). The accumulation of p-hydroxyamphetamine by brain homogenates and its role in release of catecholamines. Biochem. Pharmacol., 26: 1367-1372.

Clarke, D. E. (1980). Amphetamine and monoamine oxidase inhibition: An old idea gains new acceptance. Trends Pharmacol. Sci., July: 312-313.

Cohen, R. M., Campbell, I. C., Cohen, M. R., Torda, T., Pickar, L., Siever, J. and Murphy, D. L. (1980). Presynaptic noradrenergic regulation during depression and antidepressant drug treatment. Psychiatry Res., 3: 93-105.

Cohen, R. M., Ebstein, R. P., Daly, J. W. and Murphy, D. L. (1982). Chronic effects of a MAO-inhibiting antidepressant: Decreases in functional α-adrenergic norepinephrine-stimulated cyclic adenosine $3^1:5^1$-monophosphate systems in rat brain. J. Neuroscience, 2: 1588-1595.

Collins, G. G. S. and Sandler, M. (1971). Human blood platelet monoamine oxidase. Biochem. Pharmacol., 20: 289-296.

Cooper, J. R., Bloom F. E. and Roth, R. H. (1982). The Biochemical Basis of Neuropharmacology. Oxford Press, New York.

Coutts, R. T., Baker, G. B. and Calverley, D. G. (1980). A rapid and sensitive method of measuring meta- and para-tyramine levels in urine using electron-capture gas chromatography. Res. Commun. Chem. Pathol. Pharmacol., 28: 177-184.

Coutts, R. T., Baker, G. B., LeGatt, D. F., McIntosh, G. J., Hopkinson, G. and Dewhurst, W. G. (1981). Screening for amines of psychiatric interest in urine using gas chromatography with electron-capture detection. Progr. Neuropsychopharmacol., 5: 565-568.

Coutts, R. T., Dawson, G. W., Kazakoff, C. W. and Wong, J. Y. (1976). In vivo phenolic metabolites of N-alkylamphetamines in the rat. Evidence in favor of catechol formation. Drug Metab. Disp., 4: 256-261.

Coutts, R. T., Prelusky, D. B. and Baker, G. B. (1984). Determination of amphetamine, norephedrine, and their phenolic metabolites in rat brain by gas-liquid chromatography. J. Pharm. Sci. (in press).

Coyle, J. T. and Snyder, S. H. (1969). Catecholamine uptake by synaptosomes in homogenates of rat brains: Stereospecificity in different areas. J. Pharmacol. Exp. Ther., 170: 221-231.

Demish, L., Kaczmarczyk, P. and Gebhart, P. (1983). Methodological problems of using platelet MAO in psychiatric research. In: Modern Problems in Pharmacopsychiatry 19, Monoamine Oxidase and Its Selective Inhibitors (Beckmann, H. and Riederer, P., eds), pp. 265-277. Karger, Basel.

Dewhurst, W. G. (1968). New theory of cerebral amine function and its clinical application. Nature, 218: 1130-1133.

Donnelly, C. H. and Murphy, D. L. (1977). Substrate- and inhibitor-related characteristics of human platelet monoamine oxidase. Biochem. Pharmacol., 26: 853-858.

Dorow, R. (1982). Beta-carboline monoethylamide causes anxiety in man. Abstract from the 13th Congress of the Collegium Internationale Neuro-Psychopharmacologicum, Jerusalem, Irael.

Durden, D. A. and Philips S. R. (1980). Kinetic measurements of the turnover rates of phenylethylamine and tryptamine in vivo in the rat brain. J. Neurochem., 34: 1725-1732.

Durden, D. A., Philips, S. R. and Boulton, A. A. (1973). Identification and distribution of β-phenylethylamine in the rat. Can. J. Biochem., 51: 995-1002.

Eckhardt, S. B., Maxwell, R. A. and Ferris, R. M. (1982). A structure-activity study of the transport sites for the hypothalamic and striatal catecholamine uptake systems. Mol. Pharmacol., 21: 374-379.

Egashira, T., Ekstedt, B. and Oreland, L. (1976). Inhibition by clorgyline and deprenyl of the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol., 25: 2583-2586.

Ekstedt, B. and Oreland, L. (1975). Effect of lipid depletion on the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol., 25: 119-124.

Escobar, J. E., Schiele, B. C. and Zimmerman, R. (1974). The tranylcypromine isomers: A controlled clinical trial. Am. J. Psychiat., 131: 1025-1026.

Fowler, C. J., Tipton, K. F., MacKay, V. P. and Youdim, M. B. (1982a). Human platelet monoamine oxidase--A useful enzyme in the study of psychiatric disorders? Neuroscience, 7: 1577-1594.

Fowler, C. J., Mantle, T. J., and Tipton, K. F. (1982b). The nature of the inhibition of rat liver monoamine oxidase types A and B by the acetylenic inhibitors clorgyline, deprenyl, and pargyline. Biochem. Pharmacol., 31: 3555-3561.

Freeman, J. J. and Sulser, F. (1972). Iprindole-amphetamine interactions in the rat: The role of aromatic hydroxylation of amphetamine in its mode of action. J. Pharmacol. Exp. Ther., 183: 307-315.

Fuentes, J. A., Oleshansky, M. A., and Neff, N. H. (1976). Comparison of the apparent antidepressant activity of (-) and (+) tranylcypromine in an animal model. Biochem. Pharmacol., 25: 801-804.

Fuller, R. W. and Hemrick-Luecke, S. (1980). Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. Science, 209: 305-307.

Garrick, N. A. and Murphy, D. L. (1982). Monoamine oxidase A: Differences in selectivity towards norepinephrine compared to serotonin. Biochem. Pharmacol., 31: 4061-4066.

Gentil, V., Alevizos, B., Felix-Gentil, M. and Lader, M. (1978). Single-dose effects of tranylcypromine on psychological measures in normals. Br. J. Pharmacol., 5: 536-538.

Glassman, A. H. and Platman, S. R. (1969). Potentiation of a monoamine oxidase inhibitor by tryptophan. J. Psychiat. Res., 7: 83-88.

Gordis, C. and Neff, N. H. (1971). Monoamine oxidase: An approximation of turnover rates. J. Neurochem., 18: 1673-1682.

Gorenstein, C. and Gentil, V. (1981). Tranylcypromine isomers: Single-dose effects in normal human subjects. Psychopharmacol., 75: 400-403.

Gorrod, J. W., Lazarus, C. R. and Beckett, A. H. (1974). Some aspects of the in vitro oxidation of ^{35}S-chlorpromazine. Adv. Biochem. Psychopharmacol., 9: 191-200.

Granna, E. and Lilla, L. (1959). The inhibition of amine oxidase and the central stimulating action of the stereoisomeric amphetamines and 1-phenylethylamines. Brit. J. Pharmacol., 14: 501-507.

Green, A. L. and El Hait, M. A. S. (1980). A new approach to assessment of the potency of reversible monoamine oxidase inhibitors in vivo and its application to amphetamine, para-methoxyamphetamine and harmaline. Biochem. Pharmacol., 29: 2781-2789.

Green, A. R., Mitchell, B. D., Tordoff, A. F. and Youdim, M. B. H. (1977). Evidence for dopamine deamination by both type A and type B monoamine oxidase in rat brain in vivo and for the degree of inhibition of enzyme necessary for increased functional activity of dopamine and 5-hydroxytryptamine. Brit. J. Pharmacol., 60: 343-349.

Greiner, B. and Rommelspacher, H. (1982). Urinary metabolites of tetrahydronorharmane in the rat. In: Beta-Carbolines and Tetrahydroisoquinoline (Bloom, F., Barchas, J., Sandler, M. and Usdin, E., eds), pp. 201-208. Alan R. Liss, New York.

Grol, C. J. and Rollema, H. (1977). Conformational analysis of dopamine by the INDO molecular orbital method. J. Pharm. Pharmacol., 29: 153-156.

Grunewald, G. L., Ruth, J. H., Kroboth, T. R., Kamdar, B. V., Patil, P. N. and Salman, K. N. (1976). Conformationally defined adrenergic agents: Endo- and exo-aminobenzobicyclo (2,2,2) octenes. J. Pharmacol. Sci., 65: 920-926.

Hampson, D. R., Baker, G. B. and Coutts, R. T. (1984b). A rapid and sensitive method for quantitation of 2-phenylethylamine in brain tissue and urine. Res. Comm. Chem. Path. Pharmacol., 43: 169-172.

Hampson, D. R., Baker, G. B., Nazarali, A. J. and Coutts, R. T. (1984a). A rapid and sensitive electron-capture gas chromatographic method for the analysis of tranylcypromine in brain tissue. J. Biochem. Biophys. Methods (in press).

Hauger, R. L., Skolnick, P. and Paul, S. M. (1982). Specific [^{3}H] β-phenylethylamine binding sites in rat brain. Eur. J. Pharmacol., 83: 147-148.

Hay, J. L., McKim, H. R., Baker, G. B. and Dewhurst, W. G. (1982). Time studies on the effects of tranylcypromine plus tryptophan on formation of trypamine and 5-hydroxytryptamine in rat brain. Proc. West. Pharmacol. Soc., 25: 133-135.

Hess, S. M. and Doephfner, W. (1961). Behavioral effects and brain amine content in rats after administration of monoamine oxidase inhibitors. Arch. Int. Pharmacodyn., 134: 89-99.

Hellerman, L. and Erwin, V. G. (1968). Mitochondrial monoamine oxidase. Action of various inhibitors for the bovine kidney enzyme. J. Biol. Chem., 243: 5234-5243.

Himmelhock, J. M., Fuchs, C. Z. and Symons, B. J. (1982). A double-blind study of tranylcypromine treatment of major anergic depression. J. Nerv. Men. Dis., 170: 628-634.

Ho, B. T., Smith, R. C., Kralik, P., Allen, R., Schoolar, J., Khan, M., and DeJohn, C. (1982). Effects of neuroleptics on platelet monoamine oxidase activity. Biol. Psychiat., 17: 885-895.

Ho, B. T. and Walker, K. E. (1971). Synthesis of 1,2,3,4-tetrahydro-β-carboline. Org. Synthesis, 51: 136-138.

Hoffman, A. R., Rama Sastry, B. V. and Axelrod, J. (1979). Formation of α-methyldopamine (catecholamphetamine) from p-hydroxyamphetamine by rat brain microsomes. Pharmacology, 19: 256-260.

Holman, R. B., Elliott, G. R., Faull, K. and Barchas, J. D. (1980). Tryptolines: The role of indolamine-aldehyde condensation products in the effects of alcohol. In: Psychopharmacology of Alcohol (Sandler, M., ed.), pp. 155-169. Raven Press, New York.

Horn, A. S. and Snyder, S. H. (1972). Steric requirements for catecholamine uptake by rat brain synaptosomes: Studies with rigid analogs of amphetamine. J. Pharmacol. Exp. Ther., 180: 523-530.

Huebert, N. (1980). Formation and urinary excretion of some trace amines in the rat and in the human. Ph.D. thesis, University of Saskatchewan.

Insel, T. R., Roy, B. F., Cohen, R. M. and Murphy, D. L. (1982). Possible development of the serotonin syndrome in man. Am. J. Psychiat., 139: 954-955.

Johnston, J. P. (1968). Some observations on a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol., 17: 1285-1297.

Jones, R. S. G. and Boulton, A. A. (1980a). Interactions between p-tyramine, m-tyramine, or β-phenylethylamine and dopamine on single neurons in the cortex and caudate nucleus of the rat. Can. J. Physiol. Pharmacol., 58: 222-227.

Jones, R. S. G. and Boulton, A. A. (1980b). Tryptamine and 5-hydroxytryptamine: Actions and interactions on cortical neurons in the rat. Life Sci., 27: 1849-1856.

Kaiser, C., Trost, B. M., Beeson, J. and Weinstock, J. (1965). Preparation of some cyclopropanes and stable sulfoxoniumylides from dimethylsulfoxonium methylide. J. Org. Chem., 30: 3972-3975.

Karoum, F., Nasrallah, N., Potkin, S., Chuang, L., Moyer-Schwing, J., Philips, I., and Wyatt, R. J. (1979). Mass fragmentography of phenylethylamine, m- and p-tyramine, and related amines in plasma, cerebrospinal fluid, urine, and brain. J. Neurochem., 33: 201-212.

Kellar, K. J., Elliott, G. R., Holman, R. B., Vernikos-Danellis, J. and Barchas, J. D. (1976). Tryptoline inhibition of serotonin uptake in rat forebrain homogenates. J. Pharmacol. Exp. Ther., 198: 619-625.

Kenney, W. C., Nagy, J., Salach, J. I., and Singer, T. P. (1979). Structure of the covalent phenylhydrazine adduct of monoamine oxidase. In: Monoamine Oxidase: Structure, Function, and Altered Functions (Singer, T. P., Von Korff, R. W. and Murphy, D. L., eds), pp. 25-37. Academic Press, New York.

Kinemuchi, H., Wakui, Y., Toyoshima, Y., Hayashi, N. and Kamijo, K. (1979). PEA, a concentration-dependent preferential substrate for multiple forms of MAO. In: Monoamine Oxidase: Structure, Function, and Altered Functions (Singer, T. P., Von Korff, R. W. and Murphy, D. L., eds.), pp. 205-212. Academic Press, New York.

Kleinman, J. E., Potkin, S., Rogol, A., Buchsbaum, M. S., Murphy, D. L., Gillin, J. C., Nasrallah, H. A. and Wyatt, R. J. (1979). A correlation between platelet monoamine oxidase activity and plasma prolactin concentrations in man. Science, 206: 479-481.

Knoll, J. (1976). Analysis of the pharmacological effects of selective monoamine oxidase inhibitors. In: Monoamine Oxidase and its Inhibition, pp. 135-162. Ciba Foundation, Amsterdam.

Knoll, J. (1980). Monoamine oxidase inhibitors: Chemistry and pharmacology. In: Enzyme Inhibitors as Drugs (Sandler, M., ed.), pp. 151-171. McMillan, London.

Kueng, W., Wirz-Justice, A., Menzi, R. and Chappuis-Arndt, E. (1976). Regional brain variations of tryptophan, monoamines, monoamine oxidase activity, plasma free and total tryptophan during the estrous cycle of the rat. Neuroendocrinology, 21: 289-296.

Lang, V. A., Geibler, H. E. and Mutschlek, E. (1979). Bestimmung und vergleich der plasma-und urinkonzentrationen nach gabe von (+)- und (-)tranylcypromin. Arzneim-Forsch/Drug Res., 29: 154-157.

Langer, S. Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. Br. J. Pharmacol., 60: 481-497.

Leelavathi, D. E. and Smith, R. C. (1980). Chronic neuroleptics and brain monoamine oxidase activity. Biol. Psychiat., 15: 479-484.

Leibowitz, S. F. and Rossakis, C. (1978). Analysis of feeding suppression produced by perifornical hypothalamic injections of catecholamines, amphetamines, and mazindol. Eur. J. Pharmacol., 53: 69-81.

Leibowitz, S. F. and Rossakis, C. (1979). Mapping of brain dopamine- and epinephrine-sensitive sites which cause feeding suppression in the rat. Brain Res., 172: 101-113.

Lentzen, H. and Philippu, A. (1981). Physico-chemical properties of phenylethylamines and their uptake into synaptic vesicles of the caudate nucleus. Biochem. Pharmacol., 30: 1759-1764.

Lesse, S. (1978). Tranylcypromine (Parnate)--A study of 1000 patients with severe agitated depressions. Am. J. Psychother., 32: 220-242.

Luine, V. M. and Fischette, C. T. (1982). Inhibition of estrous behavior by intrahypothalamic implants of pargyline. Neuroendocrinology, 34: 237-244.

Luine, V. N. and Paden, C. M. (1982). Effects of monoamine oxidase inhibition on female sexual behavior, serotonin levels and type A and B monoamine oxidase activity. Neuroendocrinology, 34: 245-251.

Lyons, H. A. and Degerli, M. (1978). Indications for the use of tranylcypromine and trifluperazine (Parstelin). Scott Med. J., 23: 307-309.

Maitre, L., Delini-Stula, A. and Waldmeier, P. C. (1976). Relations between the degree of monoamine oxidase inhibition and some psychopharmacological responses to monoamine oxidase inhibitors in rats. In: Monoamine Oxidase and Its Inhibition, Ciba Foundation Symposium 39, pp. 247-270. Elsevier/Excerpta Medica/North-Holland, Amsterdam.

Martin, I. L. (1980). Endogenous ligands for benzodiazepine receptors. Trends Neurosci., 3: 299-301.

Martin, I. L. and Baker, G. B. (1977). A gas-liquid chromatographic method for the estimation of 2-phenylethylamine in rat brain tissue. Biochem. Pharmacol., 26: 1513-1516.

Matin, S. B. and Rowland, M. (1972). Electron-capture sensitivity comparison of various derivatives of primary and secondary amines. J. Pharm. Sci., 61: 1235-1240.

Maxwell, R. A., Povalsky, A. and Plummer, A. J. (1959). A differential effect of reserpine on pressor amine activity and its relationship to other agents producing this effect. J. Pharmacol. Exp. Ther., 125: 178-183.

Maxwell, R. A. and White, H. L. (1978). Tricyclic and monoamine oxidase inhibitor antidepressants: Structure-activity relationships. In: Handbook of Psychopharmacology, Vol. 14. (Iversen, L. L., Iversen, S. D. and Snyder, S. H., eds.), pp. 83-145, New York.

McKim, H. R., Calverley, D. G., Baker, G. B., and Dewhurst, W. G. (1980). The effects of tranylcypromine on the levels of some cerebral amines in rat diencephalon. In: Recent Advances in Canadian Neuropsychopharmacology (Grof, P. and Saxena, B., eds), pp. 7-13. S. Karger.

McIlwain, H. and Bachelard, H. S. (1971). Biochemistry and the Central Nervous System, 4th ed., pp. 33-54. Churchill Livingstone, London.

Meller, E., Friedman, E., Schweitzer, J. W. and Friedhoff, A. J. (1977). Tetrahydro-β-carbolines: Specific inhibitors of type A monoamine oxidase in rat brain. J. Neurochem., 28: 995-1000.

Mendelson, W. B., Buchsbaum, M. S., Murphy, D. L., Wyatt, R. J. and Gillin, J. C. (1979). Platelet monoamine oxidase and human sleep. Comm. Psychopharmacol., 2: 539-544.

Mendlewicz, J. and Youdim, M. B. H. (1983). A double-blind placebo study of L-deprenyl in affective disorders. Psychopharmacol. Bull., 19: 328-332.

Messiha, F. S., Larson, J. W. and Gellar, I. (1977). Voluntary ethanol drinking in the rat: Effects of 2-aminoethylisothiouronium salt, a modifier of NAD:NADH and noreleagine, a β-carboline derivative. Pharmacol., 15: 400-406.

Miller, H. W. and Clarke, D. E. (1978). In vitro inhibition of monoamine oxidase types A and B by d- and l-amphetamine. Comm. Psychopharmacol., 2: 319-326.

Moises, H. W. and Beckmann, H. (1981). Antidepressant efficacy of tranylcypromine isomers: A controlled study. J. Neural Trans., 50: 185-192.

Murphy, D. L. (1972). Amine precursors, amines, and false neurotransmitters in depressed patients. Am. J. Psychiat., 129: 141-148.

Murphy, D. L., Lipper, S., Slater, S. and Shilinj, D. (1979). Selectivity of clorgyline and pargyline as inhibitors of monoamine oxidases A and B in vivo in man. Psychopharmacol., 62: 129-132.

Myers, R. D. and Melchoir, C. L. (1977). Voluntary alcohol intake of tetrahydroisoquinolines or a β-carboline infused chronically in the ventricle of the rat. Pharmacol. Biochem. Behav., 7: 381-392.

Naranjo, C. (1967). Psychotropic properties of the harmala alkaloids. In: Ethnopharmacological Search for Psychotropic Drugs (Efron, D. H., Holmstedt, B. and Kline, N. S., eds.), pp. 355-391. U.S. Dept. Health, Education and Welfare, Washington, D.C.

Ninan, P. T., Insel, T. M., Cohen, R. M., Cook, J. M., Skolnick, P. and Paul, S. M. (1982). Benzodiazepine receptor-mediated experimental anxiety in primates. Science, 218: 1332-1334.

Ogren, S. O., Ask, A. L., Holm, A. C., Florvall, L., Lindbom, L. O., Lunstrom, J. and Ross, S. B. (1981). Biochemical and pharmacological properties of a new selective and reversible monoamine oxidase inhibitor, FLA 336(+). In: Monoamine Oxidase Inhibitors--State of the Art (Youdim, M. B. H. and Paykel, E. S., eds.), pp. 103-113. Wiley & Sons, Ltd., New York.

Oreland, L., Arai, Y., Strenstrom, A. and Fowler, C. J. (1983). Monoamine oxidase activity and localization in the brain and the activity in relation to psychiatric disorders. In: Modern Problems of Pharmacopsychiatry (Ban, T. A., Freyhan, P., Pichot, P. and Poldinger, W., eds), pp. 246-254. S. Karger, Basel.

Owen, F., Cross, A. J. and Lofthouse, R. (1979). Distribution and inhibition characteristics of human brain monoamine oxidase. Biochem. Pharmacol., 28: 1077-1080.

Paech, C., Salach, J. I. and Singer, T. P. (1979). Suicide inactivation of monoamine oxidase by trans-phenylcyclopropylamine. In: Monoamine Oxidase: Structure, Function, and Altered Functions (Singer, T. P., Von Korff, R. W. and Murphy, D. L., eds.), pp. 39-50. Academic Press, New York.

Paech, C., Salach, J. I. and Singer, T. P. (1980). Suicide inactivation of monoamine oxidase by trans-phenylcyclopropylamine. J. Biol. Chem., 255: 2700-2704.

Patek, D. R. and Hellerman, L. (1974). Mitochondrial monoamine oxidase: Mechanism of MAO inhibition by phenylhydrazine and arylalkylhydrazines. Role of enzymatic oxidation. J. Biol. Chem., 249: 2373-2380.

Paulos, M. A. and Tessel, R. E. (1982). Excretion of β-phenylethylamine is elevated in humans after profound stress. Science, 215: 1127-1129.

Peroutka, S. J. and Snyder, S. H. (1979). Multiple serotonin receptors: Differential binding of ^{3}H-5-hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. Mol. Pharmacol., 16: 687-699.

Philips, S. R., Baker, G. B. and McKim, H. R. (1980). Effects of tranylcypromine on the concentrations of some trace amines in the diencephalon and hippocampus of the rat. Experientia, 36: 241-242.

Philips, S. R. and Boulton, A. A. (1979). The effect of monoamine oxidase inhibitors on some arylalkylamines in rat striatum. J. Neurochem., 33: 159-167.

Philips, S. R., Durden, D. A. and Boulton, A. A. (1974). Identification and distribution of tryptamine in the rat. Can. J. Biochem., 52: 447-451.

Philips, S. R., Rozdilsky, B. and Boulton, A. A. (1978). Evidence for the presence of m-tyramine, p-tyramine, tryptamine, and phenylethylamine in the rat brain and several areas of the human brain. Biol. Psychiat., 13: 51-57.

Prelusky, D. (1983). In Vitro Models for Drug Metabolism Studies. Ph.D. thesis, University of Alberta.

Quitkin, F., Rifkin, A. and Klein, D. F. (1979). Monoamine oxidase inhibitors. A review of antidepressant effectiveness. Arch. Gen. Psychiat., 36: 749-760.

Raft, D., Davidson, J., Wasik, J. and Mattox, A. (1981). Relationship between the response to phenelzine and MAO inhibition in a clinical trial of phenelzine, amitriptyline, and placebo. Neuropsychobiology, 7: 122-126.

Raiteri, M., Angelini, F. and Levi, G. (1974) A simple apparatus for studying the release of neurotransmitters from synaptosomes. Eur. J. Pharmacol., 25: 411-412.

Raiteri, M., Bertollini, A., Angelini, F. and Levi, G. (1975). D-amphetamine as a releaser or reuptake inhibitor of biogenic amines in synaptosomes. Eur. J. Pharmacol., 34: 189-195.

Raiteri, M., Del Carmine, R., Bertollini, A. and Levi, G. (1977). Effect of sympathomimetic amines on the synaptosomal transport of noradrenaline, dopamine, and 5-hydroxytryptamine. Eur. J. Pharmacol., 41: 133-143.

Reigle, T. G., Orsulak, P. J., Avni, J., Platz, P. A. and Schildkraut, J. J. (1980). The effects of tranylcypromine isomers on norepinephrine-H^3 metabolism in rat brain. Psychopharmacol., 69: 193-199.

Riley, T. N. and Brier, C. G. (1972). Absolute configuration of (+) and (-) trans-2-phenylcyclopropylamine hydrochloride. J. Med. Chem., 15: 1187-1188.

Robinson, D. L., Campbell, I. C., Walker, M., Statham, N. J., Lovenberg, W. and Murphy, D. L. (1979). Effects of chronic monoamine oxidase inhibitor treatment on biogenic amine metabolism in rat brain. Neuropharmacology, 18: 771-776.

Robinson, D. L. and Nies, A. (1980). Demographic, biologic and other variables affecting monoamine oxidase activity. Schizophr. Bull., 6: 298-307.

Robinson, D. L., Nies, A., Ravaris, C. L., Ives, J. A. and Bartlett, D. (1978). Clinical pharmacology of phenelzine. Arch. Gen. Psychiat., 35: 629-638.

Robinson, D. L., Nies, A., Ravaris, L. C. and Lamborn, K. R. (1973). The monoamine oxidase inhibitor phenelzine in the treatment of depressive-anxiety states. Arch. Gen. Psychiat., 29: 407-413.

Rommelspacher, H. and Pannier, L. (1980). Actions of tetrahydronorharmane (tetrahydro-β-carboline) on 5-hydroxytryptamine and dopamine mediated mechanisms. Neuropharmacol., 20: 1-8.

Rommelspacher, H., Honecker, H., Barbey, M. and Meinke, B. (1979). 6-Hydroxy-tetrahydronorharmane (6-hydroxy-tetrahydro- β-carboline), a new active metabolite of indolealkylamines in man and rat. Naunyn-Schmiedeberg's Arch. Pharmacol., 310: 35-41.

Rodnight, R. (1961). Body fluid indoles in mental illness. Int. Rev. Neurobiol., 3: 263-268.

Sabelli, H. C., Fawcett, J., Gusovsky, F., Javaid, J., Edwards, J. and Jeffries, H. (1983). Urinary phenyl acetate: A diagnostic test for depression? Science, 220: 1187-1188.

Savage, D. D., Mendels, J. and Frazer, A. (1980). Monoamine oxidase inhibitors and serotonin uptake inhibitors: Differential effects on ^{3}H-serotonin binding sites in rat brain. J. Pharmacol. Exp. Ther., 212: 259-263.

Sheehan, D. V., Ballenger, J. and Jacobsen, G. (1980). Treatment of endogenous anxiety with phobic, hysterical, and hypochondriacal symptoms. Arch. Gen. Psychiat., 37: 51-59.

Shekhar, C., Mayanil, K. and Baquer, N. Z. (1982). Clorgyline and deprenyl insensitive monoamine oxidase in rat brain soluble fraction. Biochem. Pharmacol., 31: 3925-3927.

Simpson, G., White, K., Edmond, P., Razani, J., and Sloane, R. B. (1983). Monoamine oxidase and tyramine sensitivity in L-deprenyl-treated subjects. Psychopharmacol. Bull., 19: 340-342.

Singer, T. P., Edmondson, D. E. and Salach, J. I. (1981). Suicide inhibitors of central nervous system enzymes. In: Essays in Neurochemistry and Neuropharmacology, vol. 5 (Youdim, M. B. H., Lovenberg, W., Sharman, D. F. and Lagnado, J. R., eds.), pp. 131-153. John Wiley and Sons, New York.

Siris, S. G., Cooper, T. B., Rifkin, A. E., Brenner, R. and Lieberman, J. A. (1982). Plasma imipramine concentrations in patients receiving concomitant fluphenazine. Am. J. Psychiat., 139: 104-106.

Smith, D. F. (1981). Role of monoamines in behavior of reserpinized rats given tranylcypromine stereoisomers. J. Neural Trans., 50: 193-200.

Smith, D. F. and Petersen, H. N. (1982). Stereoselective effect of tranylcypromine enantiomers on brain serotonin. Life Sci., 31: 2449-2454.

Sourkes, T. L. (1979). Influence of hormones, vitamines and metals on monoamine oxidase activity. In: Monoamine Oxidase: Structure, Function, and Altered Functions (Singer, T. P., Von Korff, R. W. and Murphy, D. L., eds.), pp. 291-308. Academic Press, New York.

Squires, R. F. (1972). Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: A comparison of eight mammalian species. In: Monoamine Oxidases--New Vistas (Costa, E. and Sandler, M., eds), pp. 139-199. John Wiley and Sons, New York.

Student, A. D. and Edwards, D. J. (1977). Subcellular localization of types A and B monoamine oxidase in rat brain. Biochem. Pharmacol., 26: 2339-2341.

Sugrue, M. F. (1983). Do antidepressants possess a common mechanism of action? Biochem. Pharmacol., 32: 1811-1817.

Suzuki, O., Katsumata, Y. and Oya, M. (1979). Characterization of some biogenic amines as substrates for type A and type B monoamine oxidase. In: Monoamine Oxidase: Structure, Function, and Altered Functions (Singer, T. P., Von Korff, R. W. and Murphy, D. L., eds.), pp. 197-204. Academic Press, New York.

Tabakoff, B. and Moses, F. (1976). Differential effects of tranylcypromine and pargyline on indoleamines in brain. Biochem. Pharmacol., 25: 2555-2560.

Taylor, W. A. and Sulser, F. (1973). Effects of amphetamine and its hydroxylated metabolites on central noradrenergic mechanisms. J. Pharmacol. Exp. Ther., 185: 620-632.

Tipton, K. F. (1968). The reaction pathway of pig brain mitochondrial monoamine oxidase. Eur. J. Biochem., 5: 316-320.

Tuomisto, J. (1978). Some structural features of amine uptake mechanisms. In: Advances in Pharmacology and Therapeutics, Vol. 2 (Simon, P., ed.), pp. 231-248. Pergamon Press, Oxford.

Tuomisto, J., Tuomisto, L. and Pazdenik, T. L. (1976). Conformationally rigid amphetamine analogs as inhibitors of monoamine uptake by brain synaptosomes. J. Med. Chem., 19: 725-730.

Tyrer, J. (1976). Towards a rational therapy with monoamine oxidase inhibitors. Brit. J. Psychiat., 128: 354-360.

van Praag, H. M. (1981). Management of depression with serotonin precursors. Biol. Psychiat., 16: 291-309.

Vogel, G. W. (1983). Evidence for REM sleep deprivation as the mechanism of action of antidepressant drugs. Prog. Neuro-Psychopharmacol., 7: 343-349.

Waldmeier, P. C. and Felner, A. E. (1978). Deprenyl: Loss of selectivity for inhibition of B-type MAO after repeated treatment. Biochem. Pharmacol., 27: 801-802.

Waldmeier, P. C., Felner, A. E. and Maitre, L. (1981). Long-term effects of selective MAO inhibitors on MAO activity and amine metabolism. In: Monoamine Oxidase Inhibitors--The State of the Art (Youdim, M. B. H. and Paykel, E. S., eds.), pp. 87-102. John Wiley and Sons, New York.

Warsh, J. J., Coscina, D. V., Godse, D. D. and Chan, P. W. (1979). Dependence of brain tryptamine formation on tryptophan availability. J. Neurochem., 32: 1191-1196.

Wenger, G. R. and Rutledge, C. O. (1974). A comparison of the effects of amphetamine and its metabolites, p-hydroxyamphetamine and p-hydroxynorephedrine, on uptake, release, and catabolism of ^{3}H-norepinephrine in cerebral cortex of rat brain. J. Pharmacol. Exp. Ther., 189: 725-732.

Whaley, W. M. and Govindachari, T. R. (1951). The Pictet-Spengler synthesis of tetrahydroisoquinolines and related compounds. In: Organic Reactions (Adams, R., Adkins, H., Blatt, A. M. and Cope, A. M., eds.), pp. 151-180. Wiley, New York.

White, H. L. and Stine, D. K. (1982). Monoamine oxidases A and B as components of a membrane complex. J. Neurochem., 38: 1429-1436.

Willis, G. L. and Smith G. C. (1982). Anorexic properties of three monoamine oxidase inhibitors. Pharmacol. Biochem. Behav., 17: 1135-1139.

Wyatt, R. J., Saavedra, J. M. and Axelrod, J. (1973). A dimethyltryptamine (DMT) forming enzyme in human blood. Am. J. Psychiat., 130: 754-760.

Yasensky, D. L. W., Baker, G. B., LeGatt, D. L. and Coutts, R. T. (1982). Effects of chlorpromazine on para-hydroxylation of 2-phenylethylamine in rat liver in vitro. Proc. Can. Fed. Biol. Soc., 25: 127.

Youdim, M. B. H., Aronson, J. K., Blau, K., Green, A. R. and Grahame-Smith, D. G. (1979). Tranylcypromine overdose: Measurement of tranylcypromine concentrations and MAO inhibitory activity and identification of amphetamines in plasma. Psychological Med., 9: 377-382.

Zemlan, F. P., Ward, I. L., Crowley, W. R. and Margules, D. L. (1973). Activiation of lordotic responding in female rats by suppression of serotonergic activity. Science, 179: 1010-1011.

Zirkle, C. L., Kaiser, C., Tedeschi, D. H., Tedeschi, R. E. and Burger, A. (1962). 2-Substituted cyclopropylamines. II. Effect of structure on monoamine oxidase-inhibitory activity as measured by in vivo potentiation of tryptamine convulsions. J. Med. Chem., 5: 1265-1284.

Zivkovic, B., Guidotti, A. and Costa, E. (1974). Effects of neuroleptics on striatal tyrosine hydroxylase: Changes in affinity for the pteridine cofactor. Mol. Pharmacol., 10: 727-735.

APPENDIX I

One-way analyses of variance were employed to examine the differences between groups of samples in each experiment (e.g. 5-HT levels in control, day 1, day 10, day 21, and day 42 of TCP treatment). In the event of significant analyses of variance, pairwise comparisons between the groups in these experiments were computed using a t-test for independent means. It was also of interest to examine the relationship between two variables (e.g. 5-HT levels and TCP levels in the brain after acute and chronic administration of TCP). This was accomplished by computation of the Pearson product moment correlation coefficients which were tested for statistical significance. For all statistical procedures, two-tailed probability distributions were employed, and the general convention of $p < 0.05$ was utilized for establishment of statistical significance.

APPENDIX II

Composition of:

Incubation Mixture (reuptake and release experiments)

Concentration of Stock Solution	Volume in Final Mixture	Final Concentration in Incubation Mixture
154 mM NaCl	798.8 ml	123 mM
154 mM KCl	32.4 ml	5.0 mM
154 mM $CaCl_2$	17.6 ml	2.7 mM
154 mM $MgSO_4$	7.8 ml	1.2 mM
400 mM Tris	50.0 ml	20 mM

Add to the above 176 mg ascorbic acid, 1.8 g glucose, and 4.24 mg of nialamide. Adjust pH to 7.4 with 5 M HCl and make up to 1000 ml with distilled water.

Scintillation Fluid

677 ml	toluene
333 ml	Triton X-100
4 g	2-(4'-*tert*-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole

Phosphate Buffer

Prepare a solution of $Na_2HPO_4 \cdot 7\ H_2O$ (89.3 g/liter of distilled water) and adjust pH to 7.4 with $NaH_2PO_4 \cdot H_2O$ solution (69 g/liter of distilled H_2O).

Isotonic KCl

1.18 g KCl/100 ml distilled H_2O